9 Manufacture of Gluten-Free Specialty Breads and Confectionery Products*

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9.1 INTRODUCTION

The present chapter aims at helping the reader in the development or improvement of gluten-free bakery products by providing an understanding of the function of ingredients and processing steps. We therefore limit the formulations presented to examples, which are relevant for an understanding of the physico-chemical principles. In order to compromise between a scientifically sound piece of literature and a concise handbook for practical use, each section explains existing knowledge in the form of a critical literature review, while at the end of the sections, we summarize the most important points in a simple way. Due to our own area of work, we put some emphasis on sorghum as an ingredient, which due to some unfavorable properties of its components – like proteins firmly encapsulated in protein bodies and high starch gelatinization temperature – is especially challenging. Section 'Example: development of a gluten-free sorghum bread' (p. 154) and Table 9.3 may provide a quick introduction into the topic for those with limited time.

9.2 FORBIDDEN, PERMITTED AND CONTROVERSIAL INGREDIENTS

9.2.1 Who needs gluten-free bread?

Potential customers of gluten-free products may be three groups of people: those with celiac disease, those with wheat allergies and possibly also people who depend on a low-protein diet. Permissible ingredients will naturally depend on the target group. Most gluten-free studies focused on *celiac disease*, which is a so-called autoimmune enteropathy (Fasano and Catassi, 2001). This means that it belongs to the larger group of autoimmune diseases, in case of which the own immune system erroneously attacks components of the body. 'Enteropathy' simply means a disease of the intestine. In case of celiac disease this encompasses damage of the small intestinal mucosa, including, as a typical sign blunting or vanishing of the absorptive villi. This results – again in typical cases – in symptoms like chronic diarrhea and malabsorption. Celiac disease is induced by prolamins from wheat, rye and barley. Recent research has shown that deamidation of glutamine to glutamate by tissue transglutaminase

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within a crucial amino acid sequence plays a role, although the complete mechanism is still not known (Skovbjerg et al., 2004; Gerrard and Sutton, 2005). Celiac disease is no allergy, although sometimes erroneously called so. It is much more common than scientists assumed some decades ago, and a worldwide prevalence of 1 in 266 persons has been suggested based on modern serologic screening (Fasano and Catassi, 2001).

Typical *wheat allergies* are mediated by immunoglobulin E (IgE) and should be differentiated from other adverse reactions to wheat where IgE is not involved (Sutton et al., 1982). Sometimes adverse responses to gluten in which the immune system is not involved are termed '*gluten intolerance*' (Kadan and Schluckebier, 2007). Following this definition, gluten intolerances would exclude celiac disease and wheat allergies and be more unspecific diseases. In real *wheat allergies*, IgE antibodies may be directed against various components of wheat. Skin reactions (urticaria, i.e. hives; angioedema, i.e. swellings in the deep layers of the skin), gastrointestinal reactions (vomiting and diarrhea), asthma, hypotension (a drop in blood pressure) and in the worst case anaphylaxis (a serious, potentially life-threatening response of the whole body) may result (Sutton et al., 1982; Friedman et al., 1994; The Cleveland Clinic, 2006, *online*; WebMD, 2009, *online*). In case of the wheat-dependent, exercise-induced anaphylaxis, wheat ingestion together with physical exercise induces anaphylaxis (Lehto et al., 2003). Cross-reactions between cereals have been described (e.g. between prolamins from wheat, rye and barley; Palosuo et al., 2001).

Phenylketonuria is an example of a disease requiring a *low-protein diet*. Individuals with phenylketonuria cannot completely metabolize the essential amino acid phenylalanine, usually because of a deficiency in the enzyme phenylalanine hydroxylase. Therefore, they have unusually high plasma levels of phenylalanine, causing mental retardation via an unknown mechanism. Phenylalanine is basically present in all proteins. High protein foods like meat or dairy products are obviously a problem, but also the protein levels in normal bread are critically high (Arnold, 2007, *online*).

Summary: *Forbidden and permitted ingredients for gluten-free products depend on the target group: people with celiac disease (usually the main target group), people with wheat allergies or people on a low-protein diet.*

9.2.2 Cereals and starches

In case of celiac disease, all types of wheat, rye (*Secale cereale*) and barley (*Hordeum vulgare*) are forbidden. All three are closely related members of the grass family (Kasarda, 2001). Types of wheat include, for example, common bread wheat (*Triticum aestivum* L. ssp. *aestivum*), durum wheat (*Triticum turgidum* L. ssp. *durum*), spelt wheat (dinkel, *Triticum aestivum* L. ssp. *spelta* (L.) Thell.) and also emmer (*Triticum turgidum* L. ssp. *dicoccum*) and einkorn (*Triticum monococcum* L. ssp. *monococcum*). Since wheat and rye are forbidden, obviously also triticale is forbidden, which is a man-made cross between these two species. Occasional reports that spelt wheat might be safe for celiacs are clearly wrong, based on scientifically sound studies (Forssell and Wieser, 1995; Kasarda and D'Ovidio, 1999). Discussions about ancient, less well-known wheats like einkorn are merely of scientific interest, and it is highly recommended that celiac patients avoid them.

Safe cereals include rice, corn (maize), sorghum, millets and teff. Kasarda (2001) pointed out that only rice and corn can be regarded as safe based on scientific testing, while for example millets, sorghum and teff are just likely to be safe because they are more closely related to rice or corn than to wheat. Similarly, the pseudocereals buckwheat, amaranth and quinoa are not members of the grass family at all, and therefore only so distantly related to grasses that it is highly unlikely that they are toxic to celiacs. For practical purposes, all these grains can be regarded as safe because they have been consumed by celiacs for a long time.

Oats are probably the most controversial of all the grains. While more recent studies suggest that they are inherently safe (e.g. Picarelli et al., 2001), there remains some controversy, and often, only moderate doses, if at all, are recommended (e.g. Murray, 1999). Another concern is the contamination of oats or oat products with wheat (Thompson, 2001a). Størsrud et al. (2003) found that levels of gluten contamination in oats were in most cases low, although there was a tendency for higher levels of contamination with increased processing. In the same study, considerable gluten contamination was also detected in some samples of maize and buckwheat. This leads to the general problem of contamination. While naturally gluten-free products like rice and corn are generally regarded as safe, there is still the risk that they might be contaminated with wheat, rye or barley. Contamination might occur at all stages (growth, harvest, transport, milling and further processing) (Størsrud et al., 2003). A quality management system that includes all stages from growth to processing is therefore required. This must include obtaining gluten-free cereals or flours from reliable sources that are aware of the contamination problem. A minimum requirement is the proper separation of gluten-containing and gluten-free cereals and proper cleaning of equipment that is also used for gluten-containing cereals. Ideally, growth, transport, milling and processing should be done by specialized producers, who solely work with gluten-free materials. Regular verification of the absence of gluten, for example by ELISA (enzyme-linked immunosorbent assay) techniques, is essential.

Besides safe cereals, a variety of isolated starches can be used in gluten-free products. Within all common starches (excluding barley or rye starch), only wheat starch remains controversial. A recent study (Peräaho et al., 2003) found that the response of patients with newly detected celiac disease to a wheat-starch-based gluten-free diet was as good as to a natural gluten-free diet. However, there remains the concern of long-term damage due to small remaining amounts of gluten in wheat starch (Ciclitira et al., 1985; Thompson, 2001b) and a study by Biagi et al. (2004) with the very descriptive title 'A milligram of gluten a day keeps the mucosal recovery away: a case report' should warn us to take also small remainders of gluten seriously. Due to the fact that alternatives to wheat starch are available, there is little reason for its use, although it should also be clearly stressed that the problem of a potential gluten contamination is not limited to wheat starch.

In the case of wheat allergies, as contrasted to celiac disease, it is not possible to make a valid statement for all patients on what to avoid, due to the magnitude of components targeted by the IgE antibodies and due to possible cross-reactions. For the producer of wheat-free bread, the strict prevention of contamination, as in case of celiac disease, and clear labeling of ingredients are of central importance. We would also strongly recommend avoiding all types of wheat listed above. It has for example been shown, that spelt (*Triticum aestivum* ssp. *spelta*) triggered strong allergic reactions in a person with wheat allergy (Friedman et al., 1994).

For a low-protein diet, isolated starches have to be used, because only they guarantee very low protein levels. In contrast to celiac disease, in this case wheat starch is not a problem.

Summary: *For people with celiac disease and those with wheat allergies, all types of wheat are forbidden, and especially tricky are: for example, spelt wheat (spelt, dinkel), emmer and einkorn, which are not automatically recognized as wheat sub-species by non-scientists, but are forbidden. Anecdotal reports that, for example, spelt is tolerated by celiacs are dangerously misleading. Also forbidden for celiacs are rye, barley and triticale. Safe cereals include rice, corn (maize), sorghum, millets, teff and the pseudocereals buckwheat, amaranth* *and quinoa. Oats and wheat starch are controversial with regard to celiac disease, and we suggest a conservative approach.*

9.2.3 Other critical ingredients

9.2.3.1 *Milk*

Celiac patients often show a so-called secondary lactose intolerance. Secondary lactose intolerance is contrasted to primary lactose intolerance. The latter means that people are born without the ability to synthesize lactase and thus cannot digest lactose. In case of celiac patients, the lactase can typically be synthesized, but because it is located at the villi, it vanishes as the villi are destroyed in the course of the disease and lactose can no longer be digested. Therefore, lactose should be avoided, at least until the damage to the gut has healed due to a gluten-free diet, that is, the villi are recovered. There are, of course, some celiacs, who have at the same time primary lactose intolerance (Murray, 1996 *online*, 1999). As a consequence, milk need not be avoided generally in gluten-free bread for celiacs, but some lactose-free gluten-free products need to be available.

Milk is also a very common food allergen, ranking among the top three food allergens in various countries (Dalal et al., 2002). This might suggest avoiding it in a bread where the aim is a generally low allergenic potential.

Finally, milk is a high protein food and thus has no place in a low-protein bread.

9.2.3.2 *Egg, soy and other ingredients with allergenic potential*

Similar to milk, soy and especially egg rank high among common food allergens in various countries (Dalal et al., 2002), and both are also high protein foods, thus inappropriate for a low-protein bread. Among children, the most common food allergens are cow's milk, egg, peanut, tree nuts, soy, wheat and fish. Allergies against peanuts, tree nuts and fish/seafood usually persist lifelong (Dalal et al., 2002).

9.2.3.3 *Transglutaminase*

Transglutaminase (TGase) is an enzyme that can catalyze formation of crosslinks in proteins between lysine residues and glutamine residues. Other reactions catalyzed are the introduction of free amine groups into proteins and the hydrolyzation of glutamine residues to glutamate residues (deamidation) (Gerrard and Sutton, 2005; Fig. 9.1). Researchers have used TGase in trying to create gluten-like networks in doughs from gluten-free cereals (Gujral and Rosell, 2004; Moore et al., 2006; Renzetti et al., 2008).

As described above, deamidation of glutamine to glutamate by tissue TGase within a crucial amino acid sequence is involved in triggering celiac disease. Intuitively, this should cause concern when using microbial TGase in gluten-free products for celiacs, and similar concerns have been raised in at least two articles. Gerrard and Sutton (2005) considered the issue of TGase addition to gluten-containing cereals and expressed concern that deamidation might create the toxic epitope already during processing, thus increasing toxicity and endangering genetically predisposed individuals, whose response to gluten is dose dependent (in short: TGase might make the gluten more toxic to celiacs, making those sick who are at the edge of having overt symptoms of celiac disease). At the same time, however, Gerrard and Sutton (2005) pointed out that TGase is normally present in the human intestine. It might therefore be that there is no difference in toxicity, whether deamidation occurs during

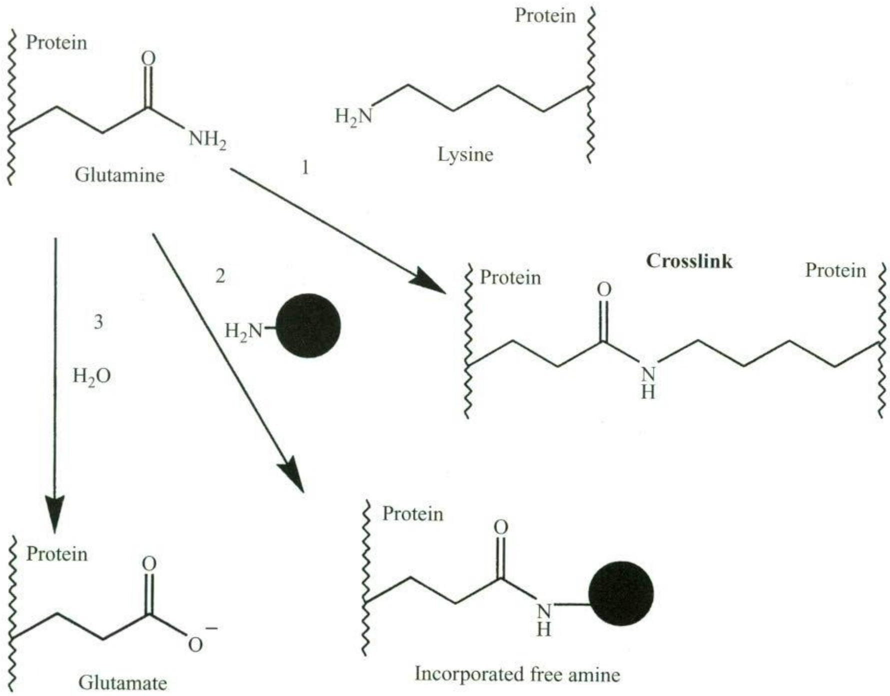


Fig. 9.1 Reactions catalyzed by transglutaminase; 1: protein crosslinking, 3: deamidation. (*Source*: Reprinted from Gerrard and Sutton (2005); copyright 2005, with permission from Elsevier.)

processing due to microbial TGase or in the intestine. These authors also mentioned that the crucial deamidation step might only be carried out by the tissue TGase in the intestine, but not by microbial TGase used in food.

Some clarification was provided by Dekking et al. (2008) and consequently, these authors adopted an even more critical position than Gerrard and Sutton (2005). Dekking et al. (2008) found that microbial TGase used in food can indeed deamidate gluten proteins, generating peptides that stimulate gluten-specific T cells from celiac patients. Microbial TGase can thus enhance immunogenicity of gluten. These authors pointed out that gluten-free foods in fact frequently and inevitably contain small amounts of gluten, so that there is no totally gluten-free diet. These small amounts of contaminants would become more toxic to celiacs due to TGase action.

The application of TGase in gluten-free bread is still in the experimental stage. Scientific studies using TGase in gluten-free bread might in fact improve the theoretical understanding of gluten-free systems. However, the above studies strongly suggest that TGase should not be used in the large-scale production of gluten-free breads destined for celiacs.

Summary: *Milk is somewhat critical for celiacs because many of them are lactose intolerant, before the damage to the gut has healed in the course of a gluten-free diet. Afterwards, it may be tolerated again. Milk, egg and soy have a high allergenic potential and are high protein ingredients. The most common food allergens for children are (cow's) milk, egg, peanut, tree nuts, soy, wheat and fish. There is increasing evidence that transglutaminase is dangerous for celiacs.*

9.3 GLUTEN-FREE BREADS

In this section, examples of bread formulations are presented, followed by a discussion of the structural principles. We start with starch breads, which are the least complex, followed by sorghum breads, rice breads and breads from other cereals/non-cereals and mixtures. We continue with some special ingredients for various types of gluten-free breads, and some more general considerations for an in-depth understanding (e.g. in how far gluten-free breads resemble rye breads). We finish the bread section with considerations on staling, which is especially important in the case of gluten-free breads, which generally stale very fast.

9.3.1 Starch-based breads

9.3.1.1 *Formulations for starch-based breads*

Table 9.1 shows different successful formulations for starch breads from the literature and two of our own experiments. Besides basic ingredients (starch, water, salt, sugar and yeast) all studies used special ingredients, that is, emulsifier (glycerol monostearate) (Jongh, 1961), soy protein isolate, xanthan gum and shortening (Ranhotra et al., 1975), xanthan gum alone (Ács et al., 1996a, b; Keetels et al., 1996; Schober and Bean, unpublished) and hydroxy-propyl methylcellulose (HPMC) alone (Schober and Bean, unpublished). The breadmaking procedures were similar amongst all studies. Ingredients were mixed, followed by a rest time, and then remixed. Final proof in bread pans and baking concluded the process.

All papers reported important findings besides the mere formulations. Jongh (1961) described that without emulsifier, the bread crumb was irregular, very coarse and hard immediately after cooling. Addition of emulsifier caused, according to the author, 'regular and reasonably fine crumb'. Further effects of the emulsifier observed were: flocculation of diluted starch suspensions (large aggregates were formed resulting in a voluminous sediment), loss of dilatancy of concentrated starch suspensions (i.e. the suspensions no longer showed an increase in viscosity with shear stress and strain), instead they became plastic and their overall consistency increased. It is worth mentioning that the emulsifier used in the study of Jongh (1961), although called glycerol monostearate, contained only 50% of this component, while 40% were distearates. We could easily bake a bread similar to the one shown by Jongh (1961) with commercial wheat starch when using active dry yeast and slightly increasing the water to 67%, even without adding emulsifier. We failed, however, when using compressed yeast and a commercial glycerol monostearate preparation, containing a minimum of 90% monoester. Ultimately, we gave up further studies on the system of Jongh (1961), because hydrocolloid-containing formulations were considerably more promising in terms of volume and crumb structure (see below and Fig. 9.2).

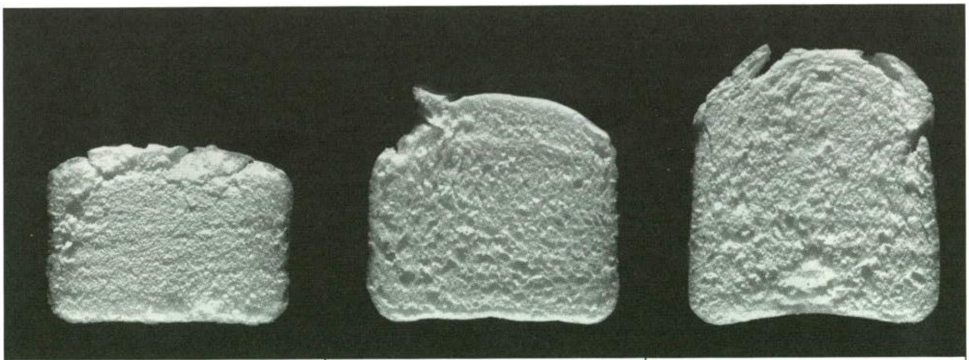
Ranhotra et al. (1975) tested the addition of different levels (0–40%) of soy protein isolate (SPI). Leavened bread with an acceptable volume (3.9 cm^3/g) could be reached without SPI, however, 20% SPI improved volume, crumb grain and texture. Without SPI, crumb was rough, crumbly and open, with SPI it was more tender, and cells were finer and more even. SPI levels higher than 20% decreased quality somewhat.

Ács et al. (1996a) compared different gums and found that xanthan gum had better effects than guar gum, locust bean gum and tragant (gum tragacanth) at any level between 1% and 5%. These authors also reported improvement of color, taste and smell by addition of 1% glucono-δ-lactone and 0.5% $NaHCO_3$.

Table 9.1 Examples of starch-based breads[a].

	Jongh (1961)	Ranhotra et al. (1975)	Ács et al. (1996a, b)[b]	Keetels et al. (1996)	Schober and Bean (unpublished) xanthan gum	Schober and Bean (unpublished) HPMC
Wheat starch	100	100	–	(100)[c]	100	100
Maize starch	–	–	100	–	–	–
Potato starch	–	–	–	(100)[c]	–	–
Water	60	155	100–140[d,e]	115	115	80
Soy protein isolate (SPI)	–	20	–	–	–	–
Glycerol monostearate	0.1	–	–	–	–	–
Xanthan gum	–	2	2	1.9	2	–
Hydroxypropyl methylcellulose (HPMC)	–	–	–	–	–	2
Salt (sodium chloride)	2	2	1.5	–	2	2
Sugar (saccharose)	4	14	5–10	1.9	4	4
Shortening/oil	–	10	–	–	–	–
Yeast[f]	6 (co[g])	7.5 (co)	6 (co)	3.8 (co)	2 (dr)	2 (dr)
Specific loaf volume (cm^3/g)	n.a.[h]	4.6	2.35[e]	2.6 (wheat) 3.3 (potato)	3.5	5.3
Critical ingredients	Wheat starch	Wheat starch, soy	None	None/Wheat starch[c]	Wheat starch	Wheat starch

[a] Best/optimized formulations, all standardized to 100 parts starch.
[b] Sensory improvements by addition of 1% glucono-δ-lactone and 0.5% $NaHCO_3$.
[c] Potato or wheat starch may be used alternatively.
[d] Water adjusted for comparable consistency (100%/140% water, when no additives/5% xanthan gum were used, respectively).
[e] Ács, E. (personal communication).
[f] Compressed (co) or active dry (dr).
[g] Yeast type not reported, compressed yeast assumed.
[h] Volume not reported; a slightly modified procedure in our own laboratory resulted in 3.9 cm^3/g (Fig. 9.2).



Following Jongh (1961), slightly modified (1650 ml, 3.9 cm^3/g)	Schober and Bean (unpublished), xanthan gum (2000 ml, 3.5 cm^3/g)	Schober and Bean (unpublished), HPMC (2490 ml, 5.3 cm^3/g)

Fig. 9.2 Three wheat starch breads (Table 9.1). The modifications relative to the original procedure of Jongh (1961) were: water increased to 68%; 6% compressed yeast replaced by 2% active dry yeast; omission of emulsifier due to the use of dry yeast.

Keetels et al. (1996) reported a lower density (i.e. higher specific volume) for potato starch bread than wheat starch bread, but a more even crumb structure for the wheat starch bread.

We added two experiments to directly compare the effect of xanthan gum and HPMC. This was done because the latter gum has been described to have much more beneficial effects in rice bread than xanthan gum (Nishita et al., 1976) and we wanted to find out, whether the same is true for starch bread. Wheat starch was used to allow comparison with the majority of studies in Table 9.1. We also repeated the experiment of Jongh (1961) in a modified way to get an idea how it compares to the more modern formulations: water was increased to 68% to achieve a smooth batter with the given wheat starch. The 6% compressed yeast was replaced by 2% active dry yeast. As described above, use of dry yeast superseded the addition of emulsifier.

The modified procedure of Jongh (1961) and both of our own experiments were carried out in a bread machine. All used an actual amount of 300 g starch per batch (Table 9.1, factor 3 for all ingredients). Duration of rest time and final proof was 15 min and 45 min, respectively, as in the study of Jongh; the bake time was prolonged to 45 min to account for the larger amount of batter. Preliminary tests indicated that too much water in combination with HPMC resulted in large holes, while insufficient water in combination with xanthan gum produced coarse bread. The formulations in Table 9.1 are based upon these findings. Figure 9.2 shows the results. The absolute volumes of the breads increased in the order Jongh (1961), Schober and Bean (xanthan gum), Schober and Bean (HPMC). In contrast, the xanthan gum bread had the lowest specific volume, lower than the bread of Jongh (1961). This is, because the xanthan gum bread contained considerably more water and therefore its weight was higher.

While the fresh bread of Jongh had a hard crumb that was leathery inside, and brittle toward the surface (Fig. 9.2, cracks), the xanthan gum bread had an elastic, soft, moist crumb when fresh, most similar to white pan bread from wheat flour. Also its pore structure resembled white wheat pan bread. The HPMC bread was very soft and fluffy, resembling cotton wool, and its volume was very high (>5 cm^3/g). In order to make the latter bread more suitable for celiacs, we tested its production from maize starch. A slight increase of the water addition to 88% on a starch basis was required, otherwise the batter was too stiff and lacked

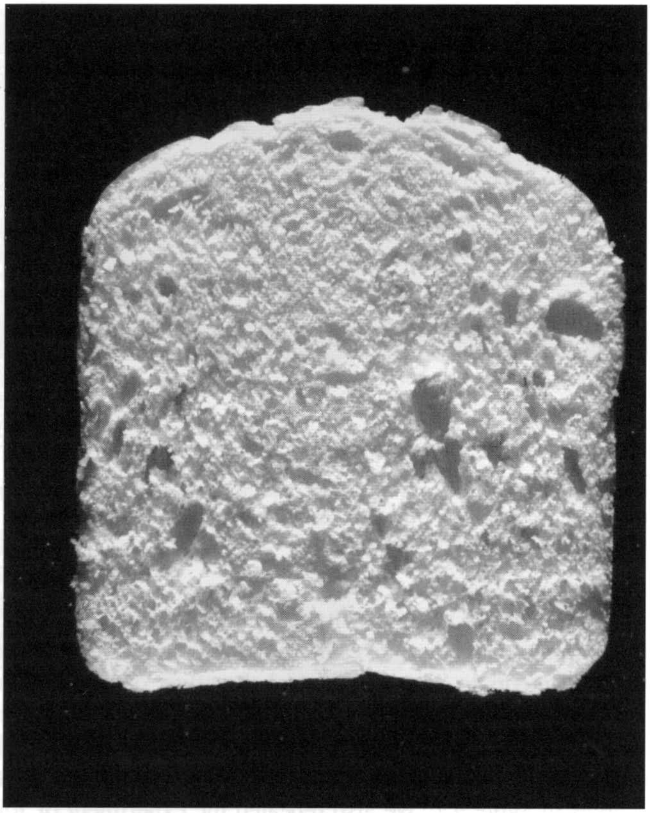


Fig. 9.3 Bread from maize starch (100%), water (88%), salt (2%), sugar (4%), active dry yeast (2%) and HPMC (2%). Specific volume was 5.3 cm^3/g.

smoothness. (Careful adjustment of the water level to reach a smooth batter, while avoiding an excess of water, appears to be a key element for bread quality.) The remaining ingredients were as described for the wheat starch-based HPMC bread. The same specific volume of 5.3 cm^3/g was reached, while the crumb was slightly coarser as for the wheat starch bread, but still good (Fig. 9.3).

In conclusion, while with pure wheat starch the greatest similarity to white pan bread could be achieved by using xanthan gum and high water addition, HPMC has the larger potential for the production of very light, highly aerated bread. The next section will address the physico-chemical background of these differences.

9.3.1.2 *Understanding the starch breads*

As always in breadmaking, there are two principally different stages: before starch gelatinization, including mixing, resting, fermenting, early stages of baking, and after starch gelatinization.

During the first stage, a simple starch batter is a suspension of starch granules and yeast cells in water, with small amounts of dissolved salt and sugar. When gas is incorporated during mixing, bubbles are suspended, which may be enlarged during fermentation. The aim is to

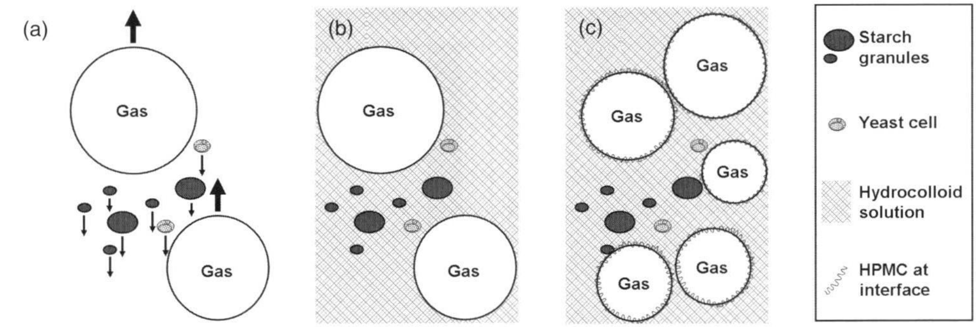


Fig. 9.4 Models for starch breads. (a) Settling of starch granules and yeast, and rising of gas bubbles, when no hydrocolloid is added. (b) Viscosity increase due to hydrocolloid addition (e.g. xanthan gum) keeps starch, yeast and gas bubbles suspended. (c) Surface-active hydrocolloids like HPMC additionally stabilize the bubbles at the gas liquid interface and prevent coalescence. A larger number of smaller bubbles results.

keep these bubbles from rising, to prevent their coalescence, and to keep the starch and yeast from settling (Fig. 9.4). A simple way to achieve this is to increase the viscosity of the liquid phase at room temperature by addition of a hydrocolloid, for example xanthan gum. Thus, rising of the bubbles and settling of the starch can be sufficiently slowed down, that the system stays homogenous during proofing, and baking until starch gelatinization. Afterwards, the starch gel provides so much viscosity that settling of remaining ungelatinized starch granules and rising of gas bubbles is no longer a problem, that is, the crumb is set. Xanthan gum differs from guar gum, locust bean gum and gum tragacanth in that its solution maintains a constant viscosity upon heating, while solutions of the latter three gums decrease in viscosity upon heating (Hoefler, 2004). This might explain that it performed best in the study of Ács et al. (1996a). The other gums would not provide sufficient viscosity during the early stages of baking when temperature increases and starch is not yet gelatinized. Additionally, locust bean gum is not completely soluble below 60°C but just swells (Hoefler, 2004).

Although it is also a hydrocolloid, HPMC differs from xanthan gum in that it is surface active (Dickinson, 2003). This originates from the hydrophobic side groups (methyl ether groups and hydroxy propyl groups) on the hydrophilic cellulose chain. A different overall substitution of cellulose with methyl ether groups and hydroxy propyl groups, and different ratios of methyl to hydroxy propyl groups can create HPMC types with different properties (Dow Chemical Company, 2005, *e-document*; Dow Chemical Company, 2007a, *online*). Surface-active substances tend to stabilize foams. They help to disperse air and thus favor formation of smaller bubbles, and they help to prevent coalescence of bubbles. Thus, in addition to increasing viscosity, HPMC also stabilizes the batter by specifically stabilizing the gas bubbles at the gas liquid interface (Fig. 9.4), and a visible consequence is the larger volume and very fluffy crumb structure of the HPMC bread (Fig. 9.2). An additional explanation is provided by one manufacturer of HPMC (Dow Chemical Company, 2007b, *online*): HPMC accumulates around the bubble surface (i.e. gas liquid interface), forming an elastic microgel there.

In order to visualize the different effects of HPMC and xanthan gum, we added 2% of the respective hydrocolloid to water and then mixed it in a blender at high speed, so that lumps were dispersed and air could be incorporated. While HPMC formed relatively stable foam almost resembling whipped egg white, xanthan gum formed a thick solution, in which only

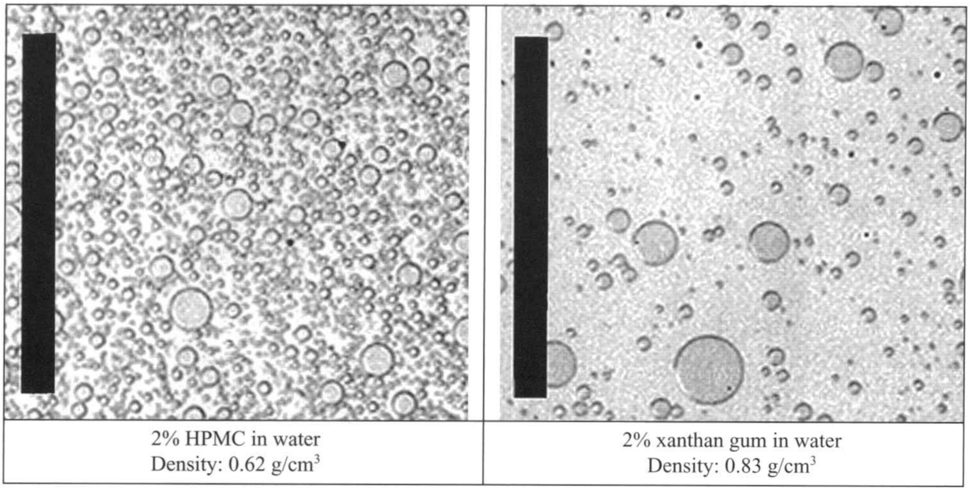


Fig. 9.5 Foams from HPMC (Methocel K4M, DOW Chemical Company, Midland, MI, USA) and xanthan gum (TICAXAN Xanthan 200, TIC Gums, Belcamp, MD, USA). Each hydrocolloid (2%) was mixed with water in a Waring-type high speed blender under standardized conditions, including 5 min rest time for swelling. For photographs, the foams were compressed between two microscope slides. Scale bars represent 5 mm. (*Source*: Reprinted from Schober et al. (2008); copyright 2008, with permission from Elsevier.)

isolated bubbles were trapped. Figure 9.5 shows the closely linked, predominantly small bubbles in HPMC foam and the individual, often large bubbles in xanthan gum, separated by wide spaces of non-aerated gum solution. Varying the xanthan gum concentration between 0.1% and 3% did not fundamentally change this behavior. At 0.1% xanthan gum, viscosity increase was too low to trap air bubbles, from 0.5% upwards, the solution/gel differed only in its viscosity/stiffness, but the coarse, distinctly separate bubbles remained. The lower density of HPMC foam (Fig. 9.5) reflected its superior aeration.

Similar to the surface activity of HPMC, other added surface-active substances may help to stabilize gas bubbles. In the case of the bread of Ranhotra et al. (1975), soy protein isolate (SPI) was added. At least two components of legume protein, 7S globulin and 11S globulin, have emulsifying properties. The 11S globulin from soybeans is called glycinin (van Vliet et al., 2002; Belitz et al., 2004). van Vliet et al. (2002) also described that glycinin forms aggregates when adsorbed at an air/water surface, which might explain that it forms a stiff protein gel layer at this interface. Ranhotra et al. (1975) could produce acceptable bread without SPI, which appears plausible because they added xanthan gum. However, the improving effects of SPI might be attributed to a stabilization of the gas bubbles at the interface, in addition to the effect of xanthan gum, which would merely keep them in suspension due to the high viscosity of the aqueous phase.

Most difficult to explain remain the results of Jongh (1961). The author claims the formation of a ‘coherent network through the whole system’, in which the starch granules are linked together at junction points, which are formed where emulsifier is adsorbed to the surface of the starch granules. The results of this starch network formation would be the previously (Section 9.3.1.1) described flocculation of starch granules, loss of dilatancy, gaining of plastic properties and improved gas holding and bread texture. However, not addressed were issues like starch-surface proteins (Seguchi and Yoshino, 1999) and their interactions with emulsifier, or the possibility that the emulsifier might just stabilize bubbles at the gas liquid interface. Bread from starch and emulsifier instead of hydrocolloid does not seem to be very important any more, in view of much better results with hydrocolloids. Nevertheless, the possibility of linking starch granules together, forming a coherent network remains an interesting hypothesis.

9.3.1.3 *Advantages and disadvantages of starch breads*

The data in Table 9.1 and the text above show that acceptable breads with good volumes can be made from pure starches. A variety of recipes resulted in acceptable bread, and the procedures were easy, avoiding lengthy procedures (like e.g. sourdough fermentation, see Section 9.3.3.2, and Table 9.2) and even avoiding somewhat difficult process steps like rounding and shaping which are common in breadmaking procedures for wheat. Principally, pure starch breads can be made in a way that they are suitable for all classes of patients described above (celiacs, people with wheat allergy, and people on a low-protein diet), when taking into account that individual ingredients (wheat starch, soy protein) might be critical.

Disadvantages include quick staling of these breads and nutritional aspects. Quick staling is a common problem of gluten-free breads, but appears to be especially unfavorable in pure starch breads (Ahlborn et al., 2005). Keetels et al. (1996) assumed that starch breads are made up from lamellae and beams which resemble concentrated starch gels in structure. Limited water during gelatinization would prevent complete swelling and disintegration of the starch granules. A small amount of amylose would leach out of the starch granules and form a thin layer between them. It appears that amylose retrogradation occurs quickly upon cooling and stabilizes the crumb (Belitz et al., 2004). According to Keetels et al. (1996), amylopectin in the swollen granules recrystallizes during storage, rendering the granules and thus the bread stiffer. Two studies (Moore et al., 2004; Ahlborn et al., 2005) suggested that continuous protein networks (either gluten in wheat bread or added egg in gluten-free bread) can mask some of the changes due to starch retrogradation. These studies will be addressed in more detail later (Section 9.3.6.1).

Obviously, starch breads lack dietary fiber, micronutrients and protein (if protein is desired). Concerning micronutrients, enrichment is possible. Dietary fiber may be added, for example in the form of inulin, which acts also as a prebiotic (Korus et al., 2006). These aspects have been discussed by Gallagher et al. (2004). A possibly less expensive and more natural way of achieving more nutritionally balanced bread is nevertheless the use of raw materials, which are less refined than starch, for example, naturally gluten-free cereals in the form of whole meal. These ingredients also contain aroma precursors (amino acids, sugars), while starch breads have a very bland flavor.

A nutritional aspect not as easily corrected as deficiencies in nutrients and fiber is the (undesirably) quick and easy availability of glucose from starch breads. Limited available literature suggests that gluten-free breads in general tend to have a higher glycemic index (GI) than regular wheat breads, that is, the blood glucose shows a larger peak during the first 2 h after consumption for gluten-free bread than for regular bread (Foster-Powell et al., 2002; Berti et al., 2004). Theoretical considerations suggest that isolation of starch granules out of a gluten or protein matrix allows easier access of amylases to the starch (Jenkins et al., 1987; Berti et al., 2004), so that in the intestine the starch can be quicker degraded to glucose, resulting in the undesirable peak in blood glucose.

Eating much food with a high GI is undesirable and involves numerous risks, including diabetes (University of Sydney, 2007, *online*). Celiac disease and type I diabetes are

Table 9.2 Examples of sorghum-based breads[a].

	Olatunji et al. (1992a)	Schober et al. (2005)	Olatunji et al. (1992b)	Hugo et al. (1997)	Hart et al. (1970)	Schober et al. (2007)
Sorghum flour	70	70	70	70	80	70
Raw cassava starch	30	–	10	10	20[b]	–
Gelatinized cassava starch	–	–	20	20	–	–
Maize starch	–	30	–	–	Alternatively[b]	–
Potato starch	–	–	–	–	Alternatively[b]	30
Water	80–100	105	100–110	90	120	105
Hydroxypropyl methylcellulose (HPMC)	–	–	–	–	2	2
Salt (sodium chloride)	1.5	1.75	1.5	2	2	1.75
Sugar (saccharose)	10	1	10	8	2	1
Shortening	1	–	1	(1)[c]		–
Emulsifier	–	–	0.6[d]	(1)[c]		–
Yeast[e]	2 (co?)	2 (dr)	1 (dr)	2 (dr)	2 (co)	2 (dr)
Extra procedures/ingredients	Fungal amylase	–	–	–	–	Maltogenic α-amylase Sourdough fermentation[f]
Specific loaf volume (cm^3/g)	2.2	1.8	2.4	3.3	n.a.	2.7

[a] Best/optimized formulations, all standardized to 100 parts sorghum flour plus starch(es).
[b] Starches from sorghum, maize, cassava, arrowroot and potato had comparable effects.
[c] Emulsifier (succinylated monoglycerides) + shortening: softer, finer crumb, increased volume, off-flavor.
[d] Monoglycerol palmitate.
[e] Compressed (co) or (instant) active dry (dr).
[f] Water, sorghum flour, 1 part skim milk powder, maltogenic α-amylase, starter (*L. plantarum*) fermented for 24 h at 30°C, then 2.4 parts of calcium carbonate added for neutralization.

associated (Fasano and Catassi, 2001; Berti et al., 2004), therefore the avoidance of food with high GI is especially important for celiacs. Thus, development of gluten-free bread from naturally gluten-free cereals instead of isolated starches is important and its production will be discussed in the following sections.

Summary: *Starch breads are the simplest gluten-free breads. The only special additive required is a hydrocolloid to prevent settling of starch granules and rising of gas bubbles during fermentation. The surface-active hydrocolloid hydroxypropyl methylcellulose (HPMC) results in larger volumes and finer, fluffier crumb than the non-surface-active xanthan gum. Nevertheless, at least in one experiment presented here, starch bread with xanthan gum resembled regular white pan bread more closely. Quick staling, high glycemic index (GI), bland flavor and lack in micronutrients and fiber are problems associated with starch breads.*

9.3.2 Breads from gluten-free cereal flours versus starch breads

9.3.2.1 *A short summary of generally recognized facts from cereal science*

The most obvious difference of any flour to an isolated starch is the variety of components in the flour, including starch, proteins, soluble and insoluble fiber (e.g. pentosans, β-glucans, cellulose), lipids, minerals and polyphenols. (In fact, many of these components are also present in commercial starch, for example lipids and proteins, but only in very little amounts.) All these components can be further sub-classified, for example, soluble versus insoluble proteins or fiber, and polar versus non-polar lipids. It is known, for example, that only polar lipids can stabilize gas bubbles. The classification and functionality of proteins, beyond their solubility, is too complex for discussing it in this short paragraph.

In isolated starch, the particle size is essentially that of the starch granules. In a flour, particle size depends on the milling technique, and on the properties of the kernel, for example its hardness. We know from wheat that durum wheat, which is very hard, yields a higher amount of coarse milling product (semolina) than bread wheat. During breadmaking, particle size may influence the speed of swelling and the speed with which soluble components are extracted from the particles into the surrounding liquid phase. Particles may also directly determine structure. For example, bran particles may disrupt uniformity of gas cells. The milling technique and kernel properties also affect the amount of mechanically damaged starch, which in turn has an effect on water-binding capacity and susceptibility to enzymes. Mechanical damage enables access of water and enzymes into the inside of the starch granules, thus increasing water binding and degradation by amylases. Milling and pretreatment before milling (like polishing or decorticating) also determines the ratio of outer layers (pericarp, seed coat, aleurone layer) and germ to endosperm and consequently the composition of the flour. As a general rule, a higher percentage of outer layers results in a higher percentage of fiber components, lipids, minerals, vitamins, polyphenols and to a certain extent proteins (aleurone proteins, not storage proteins), but in a lower percentage of storage proteins and starch. Finally, the composition of flours is influenced by genetics (variety) and environment (growth conditions) of the grain. The same variety of a grain species generally has a higher protein content, when nitrogen fertilization increases. On the other hand, there are varieties, which have higher protein contents in the same environment than others.

For details and further information, see (Belitz et al., 2004, pp. 673–746 (on general cereal chemistry); Evers and Stevens, 1985 (on starch damage); Gan et al., 1995 (on gas cell stabilization and interface effects)).

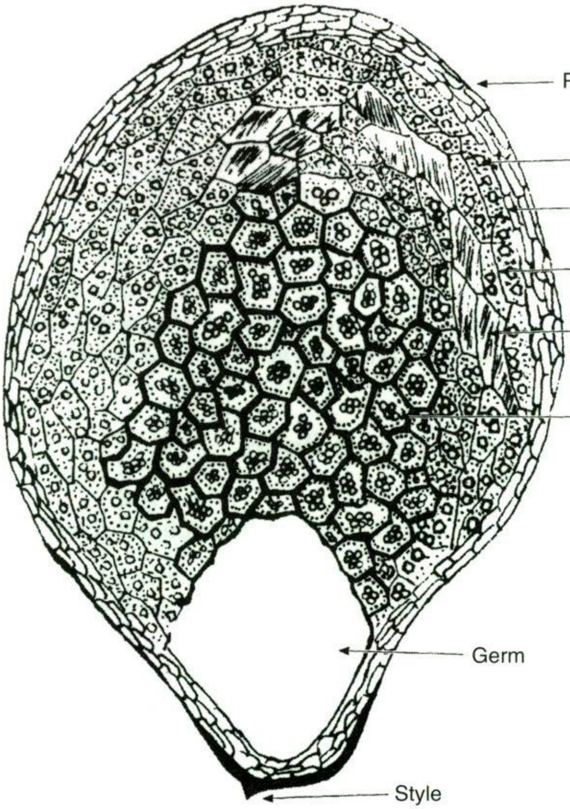


Fig. 9.6 The sorghum kernel. Mind the outer horny and inner floury endosperm. (*Source*: Reprinted from Chandrashekar and Mazhar (1999); copyright 1999, with permission from Elsevier.)

9.3.3 Sorghum-based breads

9.3.3.1 *Properties of the sorghum grain*

The sorghum kernel resembles the maize kernel in so far as it has an outer horny (corneous), vitreous and inner floury, opaque endosperm (Fig. 9.6). For practical purposes, one can assume that the outer part is at the same time vitreous and hard and the inner part opaque and soft, although Hoseney (1998) warns of a generalization in the sense that hardness and vitreousness are always the same. The horny and floury endosperm have considerable practical consequences: during initial milling, the floury part pulverizes more easily, while the horny part tends to form coarse grits (Hallgren et al., 1992). Flour from the floury part has low starch damage and tends to give less of a 'sandy' mouth feeling otherwise typical for sorghum flour (Hallgren et al., 1992). If the coarse grits from the horny endosperm are further ground into fine flour, a high amount of damaged starch results (Hallgren et al., 1992).

Protein composition is also comparable between sorghum and maize. The prolamins, called kafirins in sorghum and zeins in maize, can be further classified into α-, β- and γ-kafirins/zeins (an additional δ class is used in zeins) (Shull et al., 1991). In sorghum and maize, the prolamins (kafirins/zeins) are located in protein bodies (α-prolamins in the interior, β-, and γ-prolamins on the surface of the protein bodies) (Chandrashekar and Mazhar, 1999).

For sorghum, it has been described that the γ- and to a lesser degree β-kafirins form a disulfide-linked polymeric network that encapsulates the α-kafirins (Hamaker and Bugusu, 2003, online). Protein bodies are found in horny and floury endosperm of sorghum (Fig. 9.6) and have a diameter of roughly 1 µm. In the vitreous (horny) endosperm they are embedded in matrix protein (glutelin) (Duodu et al., 2002). Protein bodies may be relevant to technological properties in so far as they have to be disrupted in order to make the prolamins accessible. Upon cooking, sorghum proteins form disulfide-bonded oligomers, which are considerably enzyme resistant (Duodu et al., 2002). In agreement with this finding, Hamaker and Bugusu (2003, *online*) observed by laser scanning confocal microscopy that cooking causes sorghum proteins to form extended, web- or sheet-like structures with starch embedded within. Both, formation of oligomers and formation of web-like protein structures occurred to a lesser extent in maize (Duodu et al., 2002; Hamaker and Bugusu, 2003, *online*). Formation of protein aggregates upon heating may very likely affect various technological properties of sorghum.

Sorghum starch is characterized by a high gelatinization temperature, for example, relative to wheat and potato starch (Lineback, 1984), but as in all cereals, there are considerable differences between cultivars (Akingbala and Rooney, 1987).

Condensed tannins are associated by many people with sorghum. However, these polymeric polyphenols occur only in some sorghum cultivars, and it should be emphasized that virtually all sorghum cultivars grown in the US are tannin-free, for example the so-called 'white food grade sorghums', which at the same time have a white pericarp and a tan plant. The role of tannins is much more controversial than previously assumed. Tannins protect the grain against insects, weathering and birds ('bird-resistant sorghums'). Historically, focus was on nutritional disadvantages of sorghum tannins, while more recently, beneficial nutritional effects are emphasized, including a high antioxidant activity (for an overview on these aspects and further literature see Serna-Saldivar and Rooney, 1995 and Dykes and Rooney, 2006).

9.3.3.2 *Formulations for sorghum-based breads*

Table 9.2 shows formulations for sorghum-based breads from the literature. All formulations have in common that some isolated starch is used in addition to sorghum flour. Three different classes of formulations can be distinguished: Olatunji et al. (1992a) and Schober et al. (2005) added raw starch, Olatunji et al. (1992b) and Hugo et al. (1997) added mixtures of pregelatinized and raw starch, while Hart et al. (1970) and Schober et al. (2007) added HPMC in addition to raw starch. Further special ingredients were shortening and fungal amylase (Olatunji et al., 1992a), shortening and emulsifier (Olatunji et al., 1992b; Hugo et al., 1997) and maltogenic α-amylase in combination with sourdough fermentation (Schober et al., 2007).

The typical breadmaking procedure for sorghum bread was simply mixing, followed by a final proof in bread pans and baking. Olatunji et al. (1992a, b) added an additional bulk fermentation step followed by remixing before the final proof. Olatunji et al. (1992b) and Hugo et al. (1997) had to pregelatinize the starch by heating/boiling it in water. Schober et al. (2007) fermented the total sorghum flour and water together with little skim milk powder, maltogenic α-amylase and starter for 24 h at 30°C, before mixing this sourdough with the remaining ingredients, including calcium carbonate for partial neutralization.

The mentioned studies reported findings beyond the mere optimized formulations. All agreed that high water levels resulting in batters rather than doughs were required for good results, and that addition of pure starches improved results. Hugo et al. (1997) and Hart et al. (1970) furthermore reported that, while high water levels were required, excessively high levels reduced bread quality. The use of pregelatinized starch, the need for added hydrocolloids, and other improvers was controversial. With regard to pregelatinized starch, Olatunji et al. (1992b) and Hugo et al. (1997) agreed that there is an optimum ratio between raw and gelatinized cassava starch and that the gelatinzed cassava starch has the role of providing cohesiveness, viscosity and trapping air bubbles. Olatunji et al. (1992b) added that the raw cassava starch might increase strength of the system upon baking when this starch gelatinizes. In complete contrast, Hart et al. (1970) reported that no combination of pregelatinized starch, sorghum and water produced any beneficial results.

Very controversial are the studies when it comes to the use of hydrocolloids. Hart et al. (1970) tested a large range of hydrocolloids including among others gum arabic, a guar derivative, gum tragacanth, as well as different types of methylcellulose, sodium carboxy methylcellulose and HPMC (xanthan gum was not included). They identified one type of HPMC (4000 cps Methocel) that produced clearly superior results. Without hydrocolloids, the loaves collapsed upon baking, and only HPMC types provided at the same time gas retention and prevented loaves from collapsing during baking. In the studies of Olatunji et al. (1992b) and Hugo et al. (1997), pregelatinized starch could be regarded as quasi-hydrocolloid that takes up some functions of a real hydrocolloid like providing viscosity and cohesiveness. However, the findings of Olatunji et al. (1992a) and Schober et al. (2005) are in clear contrast to Hart et al. (1970) in that they show that sorghum bread can also be made on the basis of only sorghum flour and raw starch without further special additives (Fig. 9.7a). Schober et al. (2005) also tested addition of xanthan gum and found that it lowered the volume, which is in contrast to an article by Satin (1988) that reported beneficial effects of xanthan gum on sorghum bread, however without giving detailed recipes and procedures. Finally, our own preliminary tests suggested that HPMC addition delayed staling. While our first bread (Schober et al., 2005) became unacceptably stale already after 1 day, HPMC addition produced bread that was acceptable for about a week. (For antistaling effects of HPMC see also Section 9.3.8.)

With regard to fat and emulsifier, there was agreement that there are some beneficial effects of both. Hugo et al. (1997) and Hart et al. (1970) found that shortening softened the crumb, although the former authors reported that high dosage (4–5%) caused crumbliness, while the

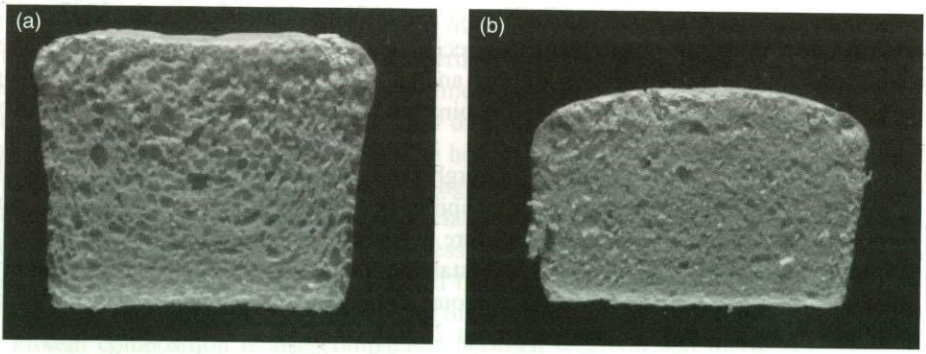


Fig. 9.7 Breads from sorghum flour (70%), maize starch (30%), water (a: 105%, b: 80%), salt (1.75%), sugar (1%) and active dry yeast (2%). Both breads were prepared from 250 g batter and proofed to height. Bread (a) reached the target height in 45 min, bread (b) was baked after 90 min (target height could not be reached, no further volume increase observed).

latter mentioned that shortening had to be combined with HPMC to prevent collapsing of the loaves. There was also agreement that some emulsifiers could soften crumb and improve crumb structure, but also weaken it or make it fragile, especially at high dosage (Hart et al., 1970; Olatunji et al., 1992b; Hugo et al., 1997). Hugo et al. (1997) furthermore reported that shortening or a combination of shortening and emulsifier reduced staling, that is, crumb firming over storage was slowed down.

Some more aspects discussed in the articles, which are relevant for an understanding of sorghum bread quality, are described as follows: Schober et al. (2005) reported overall high levels of mechanical starch damage in their sorghum flour samples. Correlations suggested that within that sample set, starch damage was higher for samples with a higher kernel hardness. On the other hand, breads from samples with higher starch damage tended to have a larger average pore size (mean cell area) but no larger volume. Schober et al. (2005) attributed the larger pore size to the fact that damaged starch is more easily degraded by amylases, resulting in more sugars for fermentation and a weaker starch gel, both resulting in a stronger expansion (and possibly coalescence) of gas bubbles. Beyond a certain point, starch damage and increase in pore size should be regarded as undesirable, especially since volume does not increase simultaneously. Two further findings by other researchers demonstrated the importance of the starch gel. Hugo et al. (1997) compared a normal, a heterowaxy (low amylose) and a waxy (virtually amylose-free) sorghum and found that the normal sorghum produced best bread, followed by the heterowaxy, while the waxy sorghum produced unacceptable bread ('with a large hole and a pudding like crumb'). The authors suggested that amylose, and possibly its retrogradation upon cooling, is critical for bread quality. Schober et al. (2007) reported that sourdough fermentation degraded proteins in the liquid phase of the batter partly. These proteins would otherwise aggregate during baking, forming strands and lumps, and interfere with the starch gel, resulting in problems like a flat top of the bread and a large hole in the crumb. Bread in which the total amount of sorghum flour had been subject to sourdough fermentation was clearly superior (Fig. 9.8).

The final question, which formulation produces the best bread, is hard to answer. Hugo et al. (1997) reported the highest specific volume, followed by Schober et al. (2007). However, for overall quality also the crumb structure plays a decisive role, and only Schober et al. (2005), Hart et al. (1970) and Schober et al. (2007) provided crumb images that would permit a direct comparison.

Summary: *Gluten-free breads can be made from sorghum flour and 20–30% pure starch, using high water levels (flour and starch to water about 1:1). A variety of successful formulations are described in the literature, but findings are sometimes contradictory between studies. Pregelatinizing part of the added starch, use of moderate amounts of certain emulsifiers, and especially use of HPMC can be beneficial. Waxy sorghum cultivars produce unacceptable bread. Damaged starch, resulting from fine milling of the horny (corneous) endosperm, has an important role on crumb structure due to its susceptibility to amylases.*

9.3.3.3 *Understanding the sorghum breads*

Viscosity increase

As in case of the starch breads, we should remember the two stages of breadmaking: before starch gelatinization, and after starch gelatinization in the course of baking. Before starch gelatinization, keeping particles and gas bubbles suspended is critical. When hydrocolloids

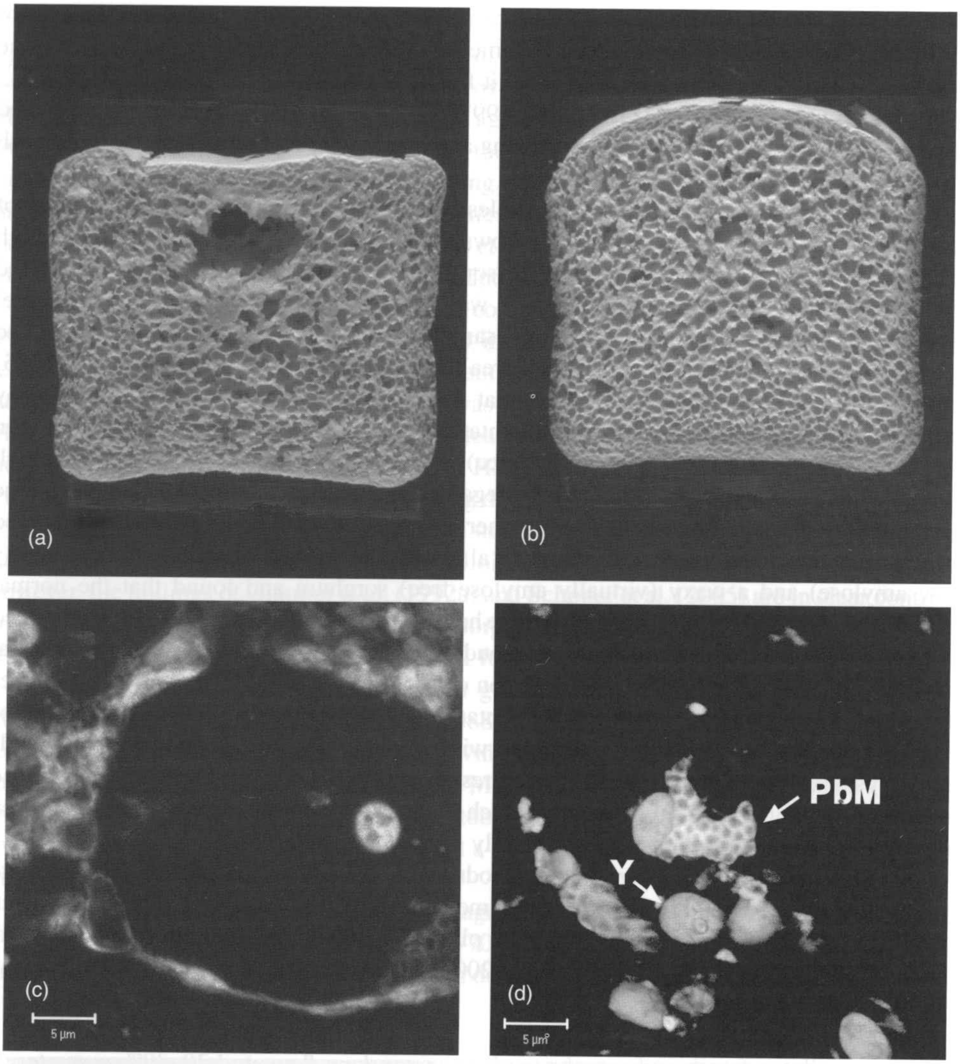


Fig. 9.8 Bread slices (a), (b) and corresponding microscopic images of the crumb (c), (d) using laser scanning confocal microscopy. (*Source*: Adapted with permission from Schober et al. (2007); copyright 2007, American Chemical Society.) Bread without sourdough (a), (c) and bread with sourdough (b), (d). In case of the sourdough bread, the total amount of sorghum flour was fermented for 24 h (formulation see Table 9.2). For the bread without sourdough, this fermentation step, as well as starter, maltogenic α-amylase and calcium carbonate were omitted. In the microscopic images, protein appears bright due to selective staining with fluorescein 5(6)-isothiocyanate. Yeast cells (Y) and protein bodies embedded in glutelin matrix (PbM) are clearly visible in the sourdough bread crumb. The crumb from bread without sourdough shows additionally strands and lumps of aggregated protein.

or pregelatinized starch are used, an increase in viscosity may be a decisive factor, as described for the starch breads (Fig. 9.4). Most interesting are however the breads without such additives (Olatunji et al., 1992a; Schober et al., 2005). In these, water levels were high and still settling of particles and rising of gas bubbles appeared to be no problem – otherwise no leavened crumb would result because the gas bubbles would rise and leave the system, and

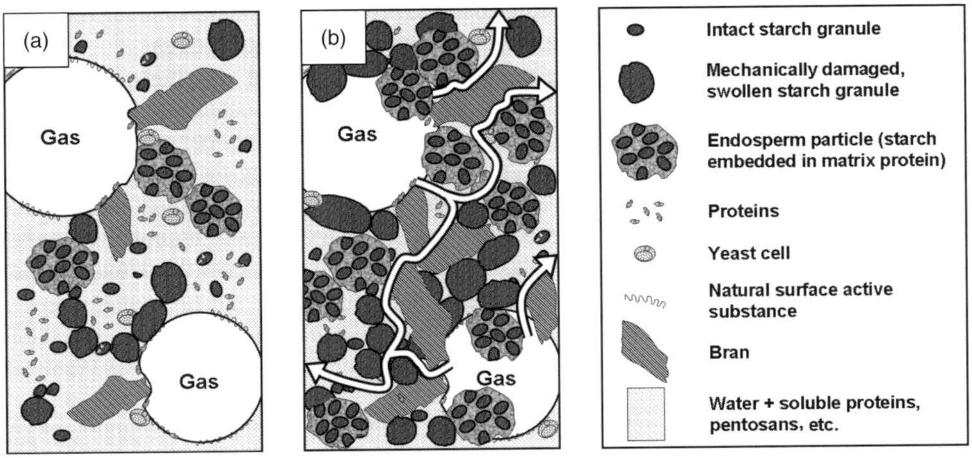


Fig. 9.9 Models for sorghum batters. (a) Thin batter with the ability to rise upon addition of extra starch and/or HPMC. (b) Thick, dough-like batter with insufficient rise.

additionally a large dense bottom layer, resulting from settled particles, could be expected in the crumb. However, although volumes were not large in these sorghum breads, and the crumb was somewhat coarse, there was still a relatively uniform leavening (Fig. 9.7a). Based on the details explained in Section 9.3.2, it is hard to draw general conclusions without having numerous analytical data on the specific flour samples used, and it is especially difficult to generalize the results from a limited sample set to a whole grain species due to differences between cultivars and growth environments. Nevertheless, data of Schober et al. (2005) in conjunction with findings of Hallgren et al. (1992) suggest that damaged starch might have a key role in at least some sorghum breads. Based on Hallgren et al. (1992), as soon as the horny (corneous) endosperm is milled into fine flour, a high amount of damaged starch will result. Likely, starch granules are so tightly packed into a rigid protein matrix in the horny endosperm (Chandrashekar and Mazhar, 1999; Duodu et al., 2002) that fine milling will break the whole matrix apart, together with the embedded starch. Accordingly, Schober et al. (2005) found very high levels of starch damage in their sample set, in which decorticated sorghum (containing horny and floury endosperm) was milled completely into fine flour on a Udy mill equipped with a small diameter screen. Interestingly, starch damage in a commercial sorghum flour was very high as well (Schober et al., 2007). Damaged starch binds more water than intact starch, and the granules swell considerably already below their gelatinization temperature (Evers and Stevens, 1985). A plausible hypothesis would therefore be that sorghum flour frequently contains numerous damaged starch granules, which at the same time bind considerable amounts of the added water and increase in size due to swelling. The result would be a sufficiently 'thick' batter, were swollen damaged starch granules and other large particles (like remaining larger endosperm particles[1] or bran) would loosely stick together, form clusters and prevent each other from settling and gas bubbles from rising by steric hindrance (Fig. 9.9a). One could compare damaged starch to pregelatinized starch in that it binds water, swells and increases viscosity at room temperature. If this hypothesis is

[1] An 'endosperm particle' would be roughly equivalent to a flour particle. We prefer using this term in order to clearly distinguish between bran particles (from the pericarp) and particles from the endosperm.

accepted, we could conclude that we either need to add pregelatinized starch or hydrocolloids, or we need to have sorghum flour with a sufficient amount of damaged starch.

Other well-known water binders like pentosans appear to be not very high in sorghum, especially if the sorghum is decorticated, because they are mainly located in the pericarp (or more simply, outer layers of the grain) (Karim and Rooney, 1972; Schober et al., 2005).

Up to here, we have discussed how to reach sufficient viscosity to keep particles and bubbles suspended. Obviously, there is also the possibility that the consistency of the sorghum batter might be too thick (all studies agreed that high water levels resulting in batters rather than doughs were required for good results, see above). We will discuss this situation later (Section 'The right balance between ingredients').

The stabilization of bubbles

Similar to added HPMC in starch breads, surface-active components in sorghum might help in the stabilization of the gas bubbles. Components to be considered in cereals in general are polar lipids, surface-active proteins (Gan et al., 1995) and water-soluble pentosans (Izydorczyk et al., 1991). Sorghum is characterized by low amounts of polar lipids due to very low amounts of glycolipids (Chung and Ohm, 2000), but still, polar lipids are present. Similarly, as stated above, sorghum is not high in pentosans, especially when decorticated. Schober et al. (2005) determined total and soluble pentosans in flours from decorticated sorghum hybrids and found very low concentrations (<0.3%) of soluble pentosans. Finally, sorghum has an albumin and globulin fraction soluble in sodium chloride solution as other grains (Jambunathan et al., 1975), which might possibly help in the stabilization of gas bubbles, but there are no details available, which would allow to verify this hypothesis.

The studies of Hart et al. (1970) and Schober et al. (2007) reported decisive improvements by the surface-active hydrocolloid HPMC (see also the section on starch breads, 9.3.1.2), while the studies by Olatunji et al. (1992b), Hugo et al. (1997) and Hart et al. (1970) reported some improvements in crumb structure by moderate levels of certain emulsifiers. Both results suggest that levels of natural surface-active components in sorghum are not high enough, so that by additional amounts of added surface-active components, bubble stabilization can be improved.

Emulsifiers, starch and crumb properties

The role of added emulsifiers in the stabilization of bubbles does not really explain why these substances tend to weaken the crumb structure at the same time, especially at high levels (Hart et al., 1970; Hugo et al., 1997). Interactions of emulsifiers with starch might provide an explanation for this phenomenon. Fatty acids, including those in organic molecules like monoglycerides, and many other small molecules may form complexes with amylose molecules, so-called helical inclusion compounds or clathrates (Hoseney, 1998; Belitz et al., 2004). Emulsifiers (surfactants) that form complexes with amylose seem to cause softer bread crumb *in regular wheat bread*, presumably because they limit starch swelling (Hoseney, 1998). Similarly, Martin et al. (1991) assumed that shortening and monoglycerides decrease starch swelling during baking. For *regular wheat bread*, these authors also argued that fewer swollen starch granules and solubilized starch might form fewer entanglements with gluten. For gluten-free bread, we might argue that under the same conditions (limited starch swelling and solubilization) fewer interactions *between* starch granules and starch molecules in solution (i.e. mainly amylose) are possible. In gluten-free batter, obviously no continuous gluten network is present that could denature during baking and thus contribute to crumb setting, so interactions within the starch phase are of central importance. The observation of Hugo et al. (1997) that waxy (i.e. practically amylose-free) sorghum produced unacceptable bread with a collapsed crumb, is in line with the important role of amylose for crumb setting (see Section 9.3.1.3). When we assume that emulsifiers and shortening limit starch swelling, and as a consequence also leaching of amylose out of the granules in the solution, there might be a trade-in between softness and crumb stability. Limited starch swelling might result in fewer interactions between starch granules and starch in solution, and therefore either in softer bread or in a weakened crumb, depending on the magnitude of the effect. We have to empirically find the type of emulsifier and shortening and the concentration level that yields the most beneficial effects. Emulsifiers and their role in staling will be discussed in Section 9.3.8.

The role of proteins and protein networks

The role of proteins in sorghum bread cannot easily be assessed in a general way. We have mentioned the *possible* contribution of soluble proteins to gas cell stabilization before. It is, however, not clear how large their role in gas cell walls really is, especially when surface-active HPMC is also used. A clearly negative effect is when proteins soluble in the liquid phase of the batter aggregate upon baking and form strands and lumps (Schober et al., 2007). It can be assumed that these aggregated proteins just interfere with the starch gel, form points of weakness, press the gel down, or reduce extensibility so that the crumb ruptures under the gas pressure and collapses, leaving a hole under the crust. We are used from wheat dough to think of protein networks as something with positive effects on bread quality. However, there is no evidence that this knowledge can be transferred to gluten-free, batter-based sorghum breads. Instead, a certain similarity to rye doughs will be discussed in Section 9.3.7.3.

The right balance between ingredients

We have discussed above how sufficient viscosity is reached in the batter, even if high water levels are added. It remains to answer the question, what the effect of low water levels, similar to those used in wheat dough, would be. Hart et al. (1970) described preliminary tests, in which sorghum dough with 35–45% moisture (dough basis, i.e. ≈54–82% water on a flour basis) did not sufficiently rise (i.e. less than double volume was reached during mixing and proofing). We made a simple experiment, in which we prepared batter from identical ingredients (70% sorghum flour, 30% maize starch, 1.75% salt, 1% sugar, 2% active dry yeast), just varying in water content. Regular water content was 105% on a flour-starch basis (formulation as in Schober et al., 2005); low water content was 80%. The regular water content produced batter of about the same consistency as pancake batter. The low water content produced a kind of dough of smooth consistency, however it lacked elasticity and extensibility when manually evaluated. The regular batter produced acceptable bread. The batter with low water hardly rose upon proofing and consequently, the resulting bread was very small and dense (Fig. 9.7).

Figure 9.9 suggests models for the breads in Fig. 9.7. Bread from thin batter (Figs. 9.7a and 9.9a) has been described above (Section 'Viscosity increase') – essentially water binding in damaged starch resulting in viscosity increase, swelling of damaged starch granules, and clusters of particles sticking together, resulting in steric hindrance of the settling of other particles and rising of gas bubbles. Obviously, there is an upper limit of how much water can be bound. When exceeding this, viscosity will be too low, and dilution of particles will be too high, so that steric hindrance is insufficient. The opposite extreme, low water-containing batters resulting in breads as in Fig. 9.7b can be modeled as in Fig. 9.9b. The solids content of such dough-like batters would be so high that numerous particles would stick together. However, unlike a gluten network, these particles would only adhere loosely via their surfaces, which can be perceived upon manual testing of the thick batter in the described lack of elasticity and extensibility. We can also assume that particles will interfere with the gas cell walls, similar as suggested by Gan et al. (1995) for doughs from whole wheat. Especially large, irregularly shaped particles like bran and endosperm particles would strongly deform the bubbles. If this deformation is only at some points, as in thin batters with sufficiently high dilution, the bubble could likely extend its surface so that it surrounds the deforming object. Too many large particles, though, would deform the bubbles at too many places, so that they would penetrate the bubble walls, resulting in leaks. Once gas starts leaking out of the bubbles, it would press its way through the weakly connected particles. There would be a point reached, when all newly formed gas would just leave the dough over existing channels formed by gas pressing the particles apart. This would explain that after little initial rise the thick, dough-like batter could no longer increase in volume upon proofing.

Table 9.3 summarizes the functionality of the constituents of sorghum flour and of the major ingredients of sorghum bread as described in this whole section (9.3.3). The information provided in Table 9.3 can help with the development of new and the improvement of existing formulations. An example for a practical use of this table is given in Section 'Example: development of a gluten-free sorghum bread' below. Prior to that, however, we want to try to address contradictions between studies.

Why are there contradictions between studies?

A lot of differences between studies may be attributed to the sorghum flour. Its starch damage will depend on the milling technique and on the sorghum grain (what is the ratio of horny to floury endosperm, how hard and how brittle are these endosperm parts?) A study working with a flour with low starch damage might more likely find that it is required to add pregelatinized starch or a hydrocolloid to promote water binding in the batter than a study using flour with high starch damage.

A simple but relevant difference between studies may be the size of the bread pans used. During proofing and the initial stage of baking before starch gelatinization, the batter is very soft. Mechanical support comes from the bottom and sidewalls of the pan, but toward the center, the batter has to support itself. When increasing the size of the pan and keeping its shape and filling level similar, volume and mass of the batter increase with the third power of the length, while the surface area only increases with the length squared. This means that in larger pans, relatively more weight has to be supported by the batter itself, while in smaller pans the support by the walls is, relatively spoken, larger. This fact is likely to facilitate collapsing of the crumb center in larger batter-based breads.

Technical preparations like hydrocolloids, emulsifiers or enzymes may vary between studies. Typically they are not chemically pure substances but mixtures of components and a certain variation between batches is inevitable. (For example, How much monoglyceride do technical monoglycerides really contain, see Section 9.3.1.1 on starch breads, or how many methyl and hydroxy propyl groups does our HPMC contain, see Section 9.3.1.2?)

Some ingredients and components of cereals may interact. For gas cell stabilization in wheat dough, a competitive mechanism between polar lipids and surface-active proteins has

Table 9.3 Functionality of ingredients before and after baking (sorghum bread used as an example).

		Before baking (batter)	**After baking (crumb)**
Sorghum	Intact starch	Binds little water	Binds much water, amylose: quick retrogradation (setting), amylopectin: slow retrogradation (staling)
	Damaged starch	Binds much water, swells strongly, degraded to sugars by amylases	Functionality depends on degree of amylolytic degradation
	Endosperm particles	Stick to each other (?), interfere with gas cells, steric hindrance	Gritty mouth feel, limit starch swelling/gelatinization (depends on size)
	Bran particles	Interfere with gas cells, steric hindrance	Gritty mouth feel, health benefits
	Insoluble proteins (protein bodies, glutelin matrix)	Little functionality	Aggregation upon heating, this may interfere with starch gel
	Soluble proteins	Possibly gas cell stabilization	Contribute to aggregation upon heating
Pure starches (not pregelatinized)	For example from maize, potato, cassava	Bind little water, dilute sorghum flour (i.e. lower percentage of damaged starch, endosperm particles, bran, proteins)	Bind much water, amylose: quick retrogradation (setting), amylopectin: slow retrogradation (staling), easily digestible (increase glycemic index)
Additives	Non-surface-active hydrocolloids (e.g. xanthan gum)	Bind much water, increase viscosity	Bind water, retain moisture in crumb during storage
	Pregelatinized starches	Bind much water, increase viscosity, susceptible to amylases	Water binding basically unchanged (unless degraded by amylases)
	Surface-active hydrocolloids (HPMC)	Stabilize gas cells, bind much water, increase viscosity	Bind water, retain moisture in crumb during storage, antistaling
	Emulsifiers	May stabilize gas cells (depends on type/concentration of emulsifier)	May affect starch retrogradation (depends on type/concentration of emulsifier), possibly antistaling
Water		Dilutes all components, reduces interactions between particles and soluble components, reduces viscosity, facilitates bubble expansion, but also coalescence and collapsing (?)	Has to be bound in set crumb mainly by gelatinized starch

been described, that is, each component alone stabilizes the gas cells better than a mixture of both (Gan et al., 1995). Similar antagonistic (or synergistic) interactions might occur in gluten-free batters between polar lipids from the grain or added emulsifiers, proteins and even HPMC. Thus, the effect of each additive would depend on the system it is used in.

Another central question is the definition (or rather feeling) for an acceptable bread. The bread in Fig. 9.7a may be rated acceptable – or not. It is obviously not very similar to white pan bread, but is leavened. It has advantages over pure starch breads (see Section 9.3.1.3), it is inexpensive, because no additives are required and it might be promoted as 'natural' and 'healthy' for the same reason. However, if we aim for a more wheat-like quality, at present the use of HPMC is the best option.

In some cases, there are even very obvious conflicts of interest between promoting health and improving quality. For example, the more starch we add the higher the resulting volume. To put it a little sarcastic: the fluffiest sorghum bread contains only added starch and HPMC, no sorghum. However, we can reasonably expect that the more starch we add the higher the resulting glycemic index (GI). To illustrate another example: higher decortication rate reduces bran and can be expected to improve volume, but with the bran, we also lose fiber and vitamins, as is well known from wheat. It could even be speculated that, if sourdough fermentation degrades proteins, as reported by Schober et al. (2007), the GI might increase, because starch is more readily accessible to amylases in the intestine. More research is required to specifically address these health aspects.

Example: Development of a gluten-free sorghum bread

(Please look at Table 9.3 for a better understanding of this example.)

Before we even start, let us think about the bread pan: It might be a good idea to use one that is not excessively large (e.g. 1 l volume or smaller), because we can then expect less trouble with a collapsing of the crumb. (More crumb weight, relatively spoken, is supported by the sides of the pan and the crust in a smaller pan). Then, there are various starting points when developing a new formulation. Let us assume that we want to keep the amount of sorghum flour as high as possible, which is desirable for a low glycemic index, so we start with 100% sorghum flour. Let us also say that we want to avoid too much trouble with bran, but also keep our bread healthy, so we might use sorghum, which has been decorticated to lose 10% of its original weight (if we can control this variable and if we do not just have to take the flour that is available). Let us then look at the crumb. We need amylose for quick retrogradation to facilitate setting. Hence, we do *not* select flour from a waxy cultivar. We also need a certain ratio of starch to water so that the crumb can set properly and is neither moist nor dry. The starch content of our sorghum flour is a given parameter. Most recipes used about 80–110% water on a flour-starch basis, so let us start with 100% and regular amounts of salt, sugar and yeast (see Table 9.2). We will now observe the consistency of our batter, which will largely depend on the amount of damaged starch – again a given parameter. If the flour has a high starch damage, the batter will be pretty thick, so we add more water (to a total of, let's say, 120% on a flour basis). When we bake our bread, we will likely encounter problems like a coarse crumb, possibly even a large hole and/or a collapsed bottom layer – both are the effects of the excessive degradation of damaged starch granules by amylases in combination with too much water. In order to dilute our damaged starch and to promote structure formation in the crumb, we add 30% pure starch (no waxy starch). We would probably not want to add pregelatinized starch if we have high starch damage, because it would bind even more water in the batter, so we add all starch raw (e.g. 70% sorghum flour, 30% ungelatinized maize, potato or cassava starch). We will now probably need less water because undamaged added starch binds less water than sorghum flour with high damaged starch content, so we can go back to about 100%. The resulting bread might look similar to Fig. 9.7a. If we are satisfied with this quality, we can finish here, and just slightly modify fermentation and baking conditions to get the best possible result out of this formulation. It is the most inexpensive, natural option.

If we aim for a higher volume, a more regular crumb structure and a slower staling rate, we might add 2% HPMC (hydroxypropyl methylcellulose e.g. Methocel K4M, DOW Chemical), which we carefully mix with our flour and starch, so that it does not form lumps upon water addition. We now have to raise the water again (e.g. to 105% or higher) – HPMC will bind more water in the batter and help in stabilizing the crumb during baking. We may further improve our bread by sourdough fermentation of the sorghum flour, in order to degrade interfering proteins. If we add amylase into our sourdough, we might even degrade undesirably high amounts of damaged starch – but we have to add an amylase that is not very thermostable (e.g. a fungal amylase), so that it does not degrade starch after it is gelatinized. We can also try a bacterial α-amylase for its antistaling effect, which will be discussed below (Section 9.3.8). And we can try emulsifiers and shortening – Table 9.2 makes some suggestions. If the bread is still not good, locate the author of this chapter and beat him up . . . this will not improve the bread, but maybe your mood.

Let us just assume the opposite of the above situation: we obtain a sorghum flour with only little starch damage. Then we need to increase our water binding, viscosity and cohesiveness of the batter. We can either add HPMC, or pregelatinized starch. If we add a total of 30% pure starch (e.g. cassava starch), we however do not want to add all of this starch in pregelatinized form. Most likely, we would otherwise run into difficulties similar to those resulting from excessive starch damage, that is, too much water bound in the batter, too much starch degraded because pregelatized starch is readily accessible to amylases, not enough starch for gelatinization and crumb setting during baking. Let's instead look at Table 9.2: 10% raw, 20% pregelatinized starch has been successfully used before. For pregelatinization, we mix part or all of the water with the starch portion to be gelatinized, boil it for some minutes and let it cool, before mixing it with the remaining ingredients. If we are about satisfied, we can make final improvements like optimizing fermentation and baking conditions.

9.3.4 Rice-based breads

9.3.4.1 *Properties of the rice grain*

Rice is harvested as so-called *paddy rice* with the hull (husk) attached. After removal of the hull, so-called *brown rice* remains. Brown rice is equivalent to the whole grain of non-hulled cereals like wheat. Removal of bran and germ from brown rice results in *white rice* (regular milled white rice, polished rice) (Hoseney, 1998; Wilkinson and Champagne, 2004). White *rice flour* can be ground from whole or broken polished kernels. As broken kernels are a by-product of polishing that sell at a lower price, rice flour is typically made from these. Brown rice flour, ground from raw brown rice, has a limited shelf life due to lipolysis by lipases (Wilkinson and Champagne, 2004; Kadan and Schluckebier, 2007).

Rice has a relatively low protein content in comparison to other cereals. Nevertheless, rice protein seems to inhibit swelling of rice starch granules (Shih, 2004). However, Hamaker and Bugusu (2003, *online*) reported that after cooking, rice proteins appeared aggregated together forming denser structures. This was in contrast to sorghum proteins, which formed

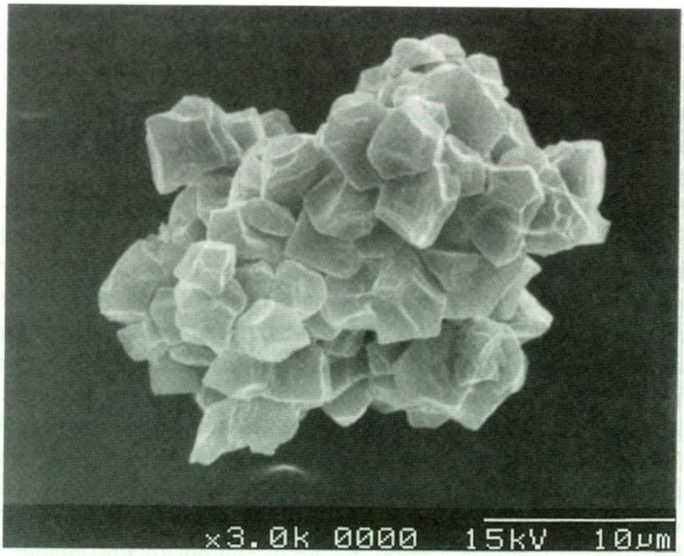


Fig. 9.10 A compound rice starch granule. (*Source*: Reprinted from Cheng and Lai (2000), with permission of the American Society for Nutrition.)

extended, web- or sheet-like structures with starch embedded within, and might indicate that the rice proteins have less negative impact on the starch gel strength and uniformity. Storage proteins in rice are located in several different types of protein bodies (diameter about 1 µm). Rice starch granules are compound, that is, very small individual starch granules with diameters of 2–4 µm aggregate to a larger unit, the compound starch granule (Fig. 9.10) (Hoseney, 1998; Champagne et al., 2004). In general, rice starch relative to wheat starch is characterized by a high gelatinization temperature similar to sorghum, and by a relatively low amylose content (Lineback, 1984). Both, gelatinization temperature and amylose content also depend on grain type (long, medium, short grain) and variety (Moldenhauer et al., 2004; Wilkinson and Champagne, 2004). As a general rule, US long grain rice varieties have higher gelatinization temperatures and amylose contents than medium and short grain types. Additionally, there is waxy (virtually amylose-free) rice (Wilkinson and Champagne, 2004). Mechanical starch damage occurs during grinding of rice to flour, and depends on the milling process (wet, semidry, or dry milling). Wet milling generally produces lower starch damage due to the cooling and lubricating effects of water, while dry milled flours, especially those with very small particle size, have high starch damage (Yeh, 2004).

It is often mentioned as an advantage that rice has a bland taste (e.g. Kadan et al., 2001; McCarthy et al., 2005). Naturally, bland taste may also be a disadvantage and be considered boring. Bland taste may be desirable for gluten-free bread when targeting groups of people who are repelled by stronger or more unusual flavors. Flours with bland taste may also be used in mixtures with ingredients that contribute intense flavors like buckwheat.

9.3.4.2 *Formulations for rice-based breads*

The suggested recipes for rice breads (Table 9.4) are comparatively uniform. In contrast to the starch and the sorghum-based breads, all used HPMC, but no other hydrocolloids or

Table 9.4 Examples of rice-based breads[a].

	Nishita et al. (1976)	Kadan et al. (2001)[b]	Kadan and Schluckebier (2007) white[b,c]	Kadan and Schluckebier (2007) brown[b,c]	McCarthy et al. (2005)	Gujral and Rosell (2004)
White rice flour	100	90.9	100	95.1	50	100
Potato starch	–	–	–	–	50	–
Rice bran (milled, defatted)	–	9.1	–	4.9	–	–
Water	75	103	110	104	79	90
Hydroxypropyl methylcellulose (HPMC)	3[d]	2.7[d]	2[d]	1.9[d]	2.2	2[d]
Salt (sodium chloride)	2	2.3	1.1	1.1	2	2
Sugar (saccharose)	7.5	12	13	12	5	7.5
Oil (vegetable, v, or rice bran oil, rb)	6 (v)	5.4 (rb)	6 (rb)	5.7 (rb)	6 (v)	6 (v)
Yeast[e]	3 (co)	2.7 (dr)	2.2 (dr)	2.1 (dr)	5 (co)	3 (co)
Extra ingredients	–	–	–	–	10 (skim milk powder)	Transglutaminase (1%, flour basis)
Specific loaf volume (cm^3/g)	5.0–5.3	1.9	4.0	4.2	3.0	2.7

[a] Best/optimized formulations, all standardized to 100 parts rice flour (plus bran or added starch if applicable).
[b] Re-calculated from weight of ingredients in published recipes, slightly rounded (Kadan and Schluckebier, 2007: % sugar appears to be miscalculated in original publication; brown rice bread re-calculated on a flour plus bran basis for the present table).
[c] Patented (USA and Kadan, 2006).
[d] Methocel K4M (Dow Chemical Company).
[e] Compressed (co) or dry (dr).

pregelatinized starch. Only one formulation used isolated starch (McCarthy et al., 2005). Possibly, the early success of Nishita et al. (1976) may have inspired successive studies. The approaches by McCarthy et al. (2005) and Gujral and Rosell (2004) are somewhat different from the rest. The former used high levels of skim milk powder, the latter focused on the effects of TGase.

The typical breadmaking procedure for rice bread does not differ from that described for sorghum bread above (Section 9.3.3.2). All studies involved mixing, final proof in bread pans and baking. Kadan et al. (2001) and Kadan and Schluckebier (2007) used home bread machines and added additional intermediate rising and punching steps between initial mixing and final proof. Similar intermediate fermentation and remixing steps have been applied by Olatunji et al. (1992a, b) in sorghum breads and are common practice in wheat bread.

Additional results reported by Nishita et al. (1976) were that other hydrocolloids besides HPMC did not produce leavened bread. Gums included were sodium carboxy methylcellulose, xanthan gum, carrageenan, locust bean gum, guar gum, gum tragacanth, gum arabic and alginates. Most other types of HPMC tested were inferior to Methocel K4M. Nishita et al. (1976) also found that water levels were critical, with insufficient water producing a stiff dough that rose only little, and excessive water causing overexpansion and thus bread loaves with large holes. Distinct decreases in volume were described when adding solid fat and emulsifiers. The reported volume (Table 9.4) is slightly ambiguous. The highest specific volume (5.3 cm^3/g) appears to have been reached without added oil. The authors reported even higher volumes (6.5 cm^3/g) for bread with 85% water, which however contained large holes. The nature of the rice flour remains also slightly ambiguous. The authors (Nishita et al., 1976) reported an ash content of 1.38%, which would be closer to what could be expected from a brown than from a white rice flour. However, they described the bread crumb as 'very white'.

The finding that excessive water produces large holes was confirmed by McCarthy et al. (2005) applying response surface methodology, but appears to be in contrast to Kadan et al. (2001) and Kadan and Schluckebier (2007), who used distinctly higher water levels than the other studies (Table 9.4). While higher water binding in batter and bread containing bran might be explained by the high water absorption of hemicelluloses in the bran, the white rice bread of Kadan and Schluckebier (2007) remains in contrast to the other studies. Possibly, the type of rice or the size of the rice flour particles might play a role. Kadan and Schluckebier (2007) defined that the majority of the flour particles should be between 100 and 150 μm. In contrast, Nishita et al. (1976) reported that 56% of their rice flour was retained by a 100 mesh sieve (i.e. in theory, 56% were larger than 149 μm). Finally, as in case of the sorghum breads, it remains difficult to compare studies. Although both Nishita et al. (1976) and Kadan and Schluckebier (2007) provided pictures of the crumb structure, a direct comparison remains difficult due to different image sizes and quality, and different loaf sizes.

Gujral and Rosell (2004) observed synergistic effects between HPMC and transglutaminase (TGase). An optimum level of TGase was found, which produced maximal specific volume and minimal crumb hardness (in their case, 1% TGase on a flour weight basis with an activity of 100 units/g). The optimal TGase level together with 2% HPMC produced the maximal volume of $\approx$2.7 cm^3/g (Table 9.4). In the absence of HPMC, volumes were very low ($\approx$1.5 cm^3/g), and only slightly improved by TGase at its optimum level. The authors put forward the hypothesis that TGase could cause the formation of a protein network that might retain carbon dioxide formed during fermentation.

All studies that included storage trials (Nishita et al., 1976; Kadan et al., 2001; Kadan and Schluckebier, 2007; and McCarthy et al., 2005) mentioned the quick staling of rice breads. Kadan and Schluckebier (2007) suggested to freeze the bread slices for storage, and to refresh them after thawing by microwave heating or toasting.

As in case of sorghum, the question for the best rice bread cannot be answered so easily. Highest volumes were achieved by Nishita et al. (1976) and the crumb structure of these breads appears acceptable, as far as can be judged from the provided photographs. When health-promoting bran is added, there appears to be a trade-in between volume and health. Low levels of about 5% can be added without adverse effects on volume as in case of the brown rice bread of Kadan and Schluckebier (2007); however, higher levels around 10% lower the volume markedly as in case of Kadan et al. (2001) (Table 9.4).

Summary: *Rice breads are typically based on white rice flour, high water levels (75–110% on a flour basis) and added HPMC. Hydrocolloids other than HPMC apparently do not work. There is no need for the addition of isolated starches. Rice bran can be added at moderate levels (e.g. 5%) without notably negative effects on bread quality, but high levels (e.g. 10%) result in low volumes. Together with starch breads, rice breads can be regarded as the 'classics' among gluten-free breads, characterized by high volumes and bland flavor. Bland flavor can be an advantage or disadvantage, depending on the customers' preferences.*

9.3.4.3 *Understanding the rice breads*

Many of the results can be interpreted with the theoretical background explained for the sorghum breads, summarized in Table 9.3. As pointed out by Hoseney (1998), rice endosperm is in general both hard and vitreous. Similar to what we discussed above (Section 9.3.3.1) for the horny endosperm of sorghum, we can expect some coarser endosperm particles and damaged starch in rice flour (depending also on milling techniques, see Section 9.3.4.1). Bran obviously is only present in brown rice flour or when it is added, as in case of Kadan and Schluckebier (2007). Rice contains insoluble and soluble proteins, and also protein bodies, similar to sorghum (Shih, 2004). Upon heating, proteins tend to aggregate together, forming denser structures, somewhat different from sorghum, where they tend to form more extended structures (Hamaker and Bugusu, 2003, *online*, see Section 9.3.4.1). Because of its different focus, the formulation of Gujral and Rosell (2004) will be treated separately in the section on TGase (Section 9.3.6.4).

Most obvious is the negative effect of bran on volume. Small amounts as in case of the brown bread of Kadan and Schluckebier (2007) may be tolerated – as hypothesized above (Section 'The right balance between ingredients'), bran particles would then deform the bubbles, but the latter could extend their surface and surround the deforming object. Large amounts of bran as in case of Kadan et al. (2001) would, however, ultimately penetrate bubble walls and cause leaks. Large amounts of bran particles could also be expected to just compress the starch gel by their weight. With these hypotheses, the slight volume increase of the brown versus the white bread of Kadan and Schluckebier (2007) cannot be explained. However, this might be simply an effect of water binding. Due to the addition of bran on top of the rice flour, the relative amount of water in the formulation decreases and additionally, bran binds water due to its hemicellulose content. (For example, insoluble pentosans swell extensively and are located mainly in the outer layers of the grain, that is bran, Belitz et al., 2004.) As in the case of sorghum, it appears that the right balance between water, hydrocolloid, damaged and intact starch has to be reached – if the resulting batter is too thick, it would not rise, if it is too thin, too high dilution would result in large holes (see Section 'The right balance between ingredients'). It has been suggested that damaged starch is undesirable for rice bread production (Yeh, 2004) and also for the patented rice bread, use of starch with low damage is recommended (USA and Kadan, 2006). However, at the same time there is a connection between milling technique, particle size and starch damage (see Section 9.3.4.1). In line with this latter hypothesis, Nishita et al. (1976) used coarser flour with presumably lower starch damage and therefore also lower water levels, while Kadan and Schluckebier (2007) used finer flour and higher water levels. It remains unclear if there is another effect of small particle size besides starch damage, like faster swelling due to better accessibility of the inside of the endosperm particles.

Similar to the balance between water and water binders, there appears to be also an optimum balance between amylose and amylopectin. In the patent (USA and Kadan, 2006), use of rice flour with about 20–26% amylose is recommended. Kadan and Schluckebier (2007) also suggested the addition of small amounts (10–20%) of waxy rice flour when certain long grain varieties are used. The patent additionally claims that waxy rice flour at the same time decreases volume but may cause softer texture. As mentioned in Section 9.3.4.1, long grain rice generally is higher in amylose. While amylose is required for crumb setting due to its quick retrogradation (Section 'Emulsifiers, starch and crumb properties' and Table 9.3), amylopectin might counteract excessive crystallinity in the crumb directly after cooling and thus cause the fresh bread to be softer.

Nishita et al. (1976) already stated that the lack of success with xanthan gum in rice breads is not understood, in view of the fact that this gum works well in wheat starch breads. We can only suggest hypotheses for an explanation. During baking, the high gelatinization temperature of rice starch would result in a longer time period, in which the bubbles expand due to heat, before they are stabilized by starch gelatinization. A surface-active hydrocolloid might help to keep the bubbles stable in this critical phase. Additionally, all breads from gluten-free flours contain coarser particles, while starch breads contain only (small) starch granules. Coarser particles would more strongly interfere with the bubbles, so that again, a surface-active hydrocolloid would be beneficial. These ideas are in line with the finding of Hart et al. (1970), who reported that basically only HPMC could be successfully used in sorghum bread, and Schober et al. (2005), who reported lack of success with xanthan gum in sorghum bread (see Section 9.3.3.2). It is important to remember the similarities between sorghum and rice in terms of high gelatinization temperature and endosperm particles.

It is interesting to note that clearly higher volumes can be reached with rice than with sorghum (Tables 9.2 and 9.4). This fact agrees favorably with the hypothesis that protein aggregation upon baking has negative effects on bread quality (see Section 'The role of proteins and protein networks'). In rice, the low flour protein content and the tendency of the proteins to just aggregate to denser structures, but not to form extended structures would then be beneficial (see Section 9.3.4.1).

9.3.5 Other cereals, pseudocereals and their mixtures

9.3.5.1 *Maize breads*

The similarity between sorghum and maize has already been pointed out (Section 9.3.3.1). Thus, it is not astonishing that Olatunji et al. (1992a) also produced a maize bread applying the identical recipe and procedure as for sorghum (70% maize flour, 30% raw cassava starch, see Section 9.3.3.2 and Table 9.2). This bread reached a slightly lower specific volume of

2.0 cm^3/g than the sorghum bread. The theoretical basis would be the same as for sorghum (Section 9.3.3.3 and Table 9.3). Very likely, the sorghum breads in Table 9.2 could all be produced also from maize with little adaptation, applying the information from Section 9.3.3 and Table 9.3.

A somewhat different type of maize breads was described by Sanni et al. (1998) and Edema et al. (2005). These so-called sour maize breads were made from maize flour and maize starch (70:30) in case of Sanni et al. (1998) and different maize flours, soy flours and blends of maize (80–90%) and soy (10–20%) in case of Edema et al. (2005). In both studies, leavening was achieved with mixed cultures of lactic acid bacteria and yeast, and salt, fat, sugar and high water levels were added. In case of Sanni et al. (1998), in addition one egg per 100 g flour was used. Specific volumes of these breads were very low (<1 cm^3/g). The authors emphasized that these breads are specialty breads, and have advantages like improved mold-free shelf life relative to yeast leavened breads, or improved protein quality in nutritional terms when maize–soy mixtures are used.

9.3.5.2 *Mixtures*

Various mixtures of gluten-free cereals, pseudocereals and other ingredients like beans have either been described in the literature or are commercially available for bread production. The more complex the mixture, the more difficult is the understanding of the contribution of each component. We will therefore limit this section to a few examples, and the explanation of only the most obvious facts.

Sanchez et al. (2002) optimized a bread formulation from corn starch, rice flour and cassava starch, with and without soy addition, applying response surface methodology. The optimum formulation contained 74% corn starch, 17% rice flour and 9% cassava starch (100% total flour), plus 0.5% soy flour. Other ingredients comprised an undisclosed gum, salt, sugar, fat, yeast and 83–100% water on a total flour basis. As expected, soy improved crumb structure, presumably due to surface-active components like glycinin (see Section 9.3.1.2). With regard to the effect of the starches and rice flour, different gelatinization temperatures (maize and rice: high, cassava: low), particle sizes and starch damage might explain the existence of an optimum.

Moore et al. (2004) developed and studied two gluten-free bread formulations from a variety of ingredients. The non-dairy (ND) formulation had a flour-starch basis of brown rice flour (25%), corn starch (54%), buckwheat flour (8.5%) and soy flour (12.5%). Other ingredients were xanthan gum, salt, sugar, sugar syrup, yeast and 105% water on a flour-starch basis. We can see that only a limited amount of buckwheat flour was used, reflecting its intense flavor. Furthermore, soy flour was added, which likely contributed to gas cell stabilization, and the flours were diluted by $>50\%$ pure starch (compare Table 9.3). Xanthan gum contributed viscosity. This bread staled faster than a wheat bread control, as detected especially by a much more dramatic drop in cohesiveness over 5 days of storage. The second formulation (dairy bread, D) in the same study showed a better keeping quality, especially distinctly less loss of cohesiveness than the ND formulation. This indicates that the dairy bread became less brittle over storage. Its flour-starch basis was brown rice flour (50%), potato starch (25%), corn starch (12.5%) and soy flour (12.5%). A very high amount of 37.5% skim milk powder on a flour-starch basis was included. Other ingredients were xanthan gum, konjac gum, salt, sugar, yeast, baking powder, a considerable amount of egg (30% fresh whole egg on a flour-starch basis) and water (105%). For this bread, a lower amount of pure starches was added relative to the ND bread, while stabilizing factors included the two gums, soy and egg. The effects of skim milk powder are not clear. Both, egg and skim milk powder require a separate, in depth discussion (Sections 9.3.6.1 and 9.3.6.2).

A formulation somewhat comparable to the dairy bread by Moore et al. (2004) was developed by Ahlborn et al. (2005). The flour-starch basis of their gluten-free rice bread was white rice flour (70%), tapioca (cassava) flour (13%) and potato starch (17%). Stabilizing ingredients were fresh whole egg (17% on a flour-starch basis), xanthan gum and HPMC. Other ingredients were salt, sugar, yeast, oil, skim milk powder (4%) and water. This gluten-free rice bread showed less staling than wheat and low-protein starch breads, measured as resistance to mechanical collapse in two uniaxial compression cycles.

Many commercial gluten-free bread mixes or mixes described in cookbooks for celiacs contain bean flours, namely garbanzo bean (chickpea, *Cicer arietinum*) and fava bean (broad bean, *Vicia faba*) flours (Fenster, 2004; Bob's Red Mill, 2008, *online*). A bean flour mix suggested by Fenster (2004) contained over 50% of bean flour, and from the position of garbanzo bean flour in the list of ingredients one can conclude that also many commercial mixes contain substantial amounts (Bob's Red Mill, 2008, *online*). The mentioned beans are members of the *Fabaceae* family (legumes), as are soybeans (Belitz et al., 2004). We have mentioned the surface activity of legume proteins before (7S and 11S globulins, see Section 9.3.1.2). However, the findings of Sanchez et al. (2002) reported in the present section suggest that low amounts (<1%) of soy flour are sufficient for an improvement of crumb structure. Other reasons for addition of large amounts of legumes would be their high content in protein and dietary fiber (Belitz et al., 2004). It is also important that proteins from legumes and cereals supplement each other in their biological value, as lysine is the limiting amino acid in cereals, while methionine is the limiting amino acid in beans (Hegarty, 1995; Belitz et al., 2004). Simplified, the combination of cereal protein and bean protein is 'healthier' or 'more useful' for the body than each individual protein, as the combination contains a more favorable mixture of essential amino acids.

Similar to legumes, the pseudocereals amaranth, quinoa and buckwheat may be added to gluten-free products in order to improve the nutritional value. The favorable amino acid composition of pseudocereals has been pointed out by Kuhn (1999) and Kuhn et al. (2000). The USDA National Nutrient Database for Standard Reference (USDA, 2008, *online*) shows lysine contents for all three pseudocereals of 0.6–0.8 g/100 g versus 0.3–0.4 g/100 g for whole wheat.

Summary: *Formulations for gluten-free breads from mixtures of a variety of ingredients have been described in the literature. Similar to commercial gluten-free bread mixes, the contribution of each individual ingredient is not always easily understood. Examples for ingredients used in mixtures are corn starch, potato starch, cassava starch, white and brown rice flour, soy flour, buckwheat flour, skim milk powder, egg, bean flours (chickpea and broad bean), xanthan gum, konjac gum and HPMC. Reasons for the addition of an individual ingredient may be technological (e.g. in case of gums or egg the improvement of the crumb structure), sensory (e.g. buckwheat, in case of which small amounts may add a more intense flavor, but large amounts may taste too strong) or nutritional (e.g. bean flours add protein and fiber, and the amino acid composition supplements with cereals resulting in an increased biological value of the protein). Pseudocereals (amaranth, quinoa, buckwheat) may also be added for their nutritional value.*

9.3.6 Special ingredients and additives for gluten-free bread

9.3.6.1 *Egg products*

Important functions of egg are surface activity and emulsifying properties of egg white and yolk, and heat coagulation of egg white (Forsythe, 1970; Satin, 1988; Cauvain, 1998). From these properties we can expect that egg addition to gluten-free bread helps in the stabilization of the gas cells due to its surface activity as well as in the setting of the crumb due to heat coagulation of the egg white. These effects are well known from cake making, and indeed such breads have some similarity with cakes (*personal observation*).

The studies of Moore et al. (2004) and Ahlborn et al. (2005) agreed that formulations with egg produced breads with delayed staling (see previous Section 9.3.5.2). Both found web- or film-like structures resembling gluten in crumb from gluten-free breads containing egg, but not in egg-free gluten-free formulations. In line with the theory, these structures were likely denatured egg white. Both studies hypothesized that these protein matrices were the factor counteracting staling, for example, by simply masking some of the changes originating from starch retrogradation. A trade-in for the delayed staling upon egg incorporation is that we add an ingredient with allergenic potential (see Section 9.2.3.2). Egg-containing breads might therefore be better regarded as specialty than as a mainstream gluten-free product.

9.3.6.2 *Milk products*

For wheat breads, it is generally assumed that milk products like skim milk, casein, whey and buttermilk have beneficial effects like increasing the water-binding capacity of the dough and the moistness of the crumb due to the increased protein content (Belitz et al., 2004). It should, however, not automatically be assumed that the same is true for gluten-free breads. In the absence of a gluten-network, the gas cell stability and strength of the starch gel become more relevant. The right balance between water binding in the batter and water binding after baking appears to be the relevant factor rather than increasing water absorption, and it has already been described for sourdough fermentation of sorghum that protein degradation was beneficial (see Section 9.3.3.3 and Table 9.3).

Gallagher et al. (2003) examined the effects of different dairy powders added to a starch-based commercial gluten-free flour. Included were types of whey, skim milk powder and other milk solids, sodium caseinate, and milk protein isolate, ranging between 6.5% and 90% protein, in levels of 3%, 6% and 9% on a flour weight basis. Overall, these powders reduced loaf volume, although there were differences between type of powder and addition levels. Dairy powders improved crust browning and in some cases also softness of the crust. Both of these effects may be regarded as desirable. However, all dairy powders reduced crumb softness. Increasing the water content could increase volume and crumb softness of dairy-containing breads. Sensory results pointed toward a higher acceptability of the dairy breads. Nutritionally, dairy powders increase protein content, while those with high lactose content can be problematic for celiac patients with a secondary lactose intolerance. Milk has also an allergenic potential (see Section 9.2.3.1).

The results of a response surface study on sorghum bread by Schober et al. (2005) were more critical with regard to the technological effects of skim milk powder. Skim milk powder decreased loaf height by causing a collapsed top of the breads and reduced crumb cohesiveness. Only improved crust browning was found to be a positive effect.

Latest approaches involve the use of a casein, at specific pH and ionic strength, in order to form gluten-like masses (Gallagher, 2006, *online*).

9.3.6.3 *Other animal products*

Other animal proteins that have been suggested for use in gluten-free breads are fish surimi (Gallagher et al., 2004) and gelatin (Kieffer, R., German Research Center for Food Chemistry, Garching, Germany, *personal communication*). Surimi is muscle protein from fish that has been water washed. Together with water, it forms a solid cohesive gel (Belitz et al., 2004). Gelatin is extracted from animal bones or skin under acid or alkaline conditions. Like surimi, it is a gelling agent (Belitz et al., 2004; Hoefler, 2004). In contrast to what might possibly be expected, Gallagher et al. (2004) reported that taste panels could generally not detect a difference between control and surimi breads. At the same time, loaf volume and crust and crumb softness were improved by addition of most types of surimi. It remains, however, questionable whether consumers would accept a bread made with fish or gelatin, even if they could not taste any off-flavor.

Summary: *Egg has doubtlessly technological benefits in gluten-free bread, while the role of milk products is more controversial. Both have allergenic potential, and lactose from milk is critical for celiacs when starting the gluten-free diet as long as the damage to the intestine has not healed (see Section 9.2.3.1). Both should therefore be used with care and have to be clearly labeled.*

9.3.6.4 *Antistaling α-amylases*

Some α-amylases are very promising for delaying the staling of gluten-free bread. Details about these enzymes will be discussed in the section on staling (Section 9.3.8).

9.3.6.5 *Transglutaminase*

The basic idea for the use of TGase in gluten-free bread is very straightforward. The main reaction catalyzed by this enzyme is the formation of new covalent crosslinks in proteins via lysine and glutamine residues (see Section 9.2.3.3 and Fig. 9.1). The proteins in all gluten-free cereals do not aggregate to continuous networks in the dough at room temperature, unlike wheat gluten. Therefore, it appears logical to artificially crosslink them and thus create a network. However, up to now, it appears that no attempt has lead to a gluten-like product.

Moore et al. (2006) added protein sources (12.5% of soy flour, skim milk powder, or whole egg powder) to a gluten-free base mix from white rice flour (35%), potato starch (30%) and corn starch (22.5%). Other ingredients were salt, yeast, sugar, xanthan gum and water. The study aimed at how these protein sources, rather than flour protein, would be crosslinked by different levels of TGase. Bread volume was affected by the type of protein source. Egg addition resulted in higher volumes than the other protein sources, in line with the theoretical background (see Section 9.3.6.1). TGase had little effect on volume, except that at its highest dosage in combination with skim milk powder, bread volume was lowered. These results suggest that TGase has no beneficial effects on gas holding capacity in combination with any of these protein sources. The authors observed network formation in the protein phase due to the added TGase in the systems with skim milk powder and egg powder. It appears however that these networks did not improve bread quality.

The study of Gujral and Rosell (2004) has already been described above (Section 9.3.4.2) and in Table 9.4. In contrast to Moore et al. (2006), no protein source was added, but the effect of TGase directly on rice proteins studied. Several variables indicated that rice proteins were indeed crosslinked: with increasing levels of TGase, free amino groups decreased. Rheological measurements (dynamic oscillatory frequency sweeps) showed an increase in elastic and viscous modulus with increasing levels of TGase, indicating more resistance to deformation at small shear deformations. Increasing levels of TGase also increased farinograph consistency. However, bread with acceptable volume could only be produced, if HPMC was added in addition to TGase. Therefore, the protein crosslinking achieved with TGase most likely did not produce a network with properties similar to gluten. More research would be required to understand, how exactly the bread volume was improved by the combination of TGase and HPMC.

Finally, Renzetti et al. (2008) studied the effect of TGase in formulations from different cereal and pseudocereal flours (buckwheat, brown rice, oat, sorghum, teff and corn flour), with only water, yeast, salt and sugar added. Due to the absence of added hydrocolloids, this is an especially challenging system. As in the case of Gujral and Rosell (2004), cereal proteins rather than added protein sources were the target for TGase action. The authors found that the effect of TGase differed between the studied cereals. In buckwheat and brown rice, increasing levels of TGase caused a decrease in specific volume. In sorghum and corn, low levels of TGase increased the specific volume. However, all specific volumes were low (<2.2 ml/g) and in sorghum and corn, the specific volume was improved by TGase to only 1.6–1.7 ml/g. The most remarkable positive effect of TGase was an improvement of the crumb structure of buckwheat, brown rice and corn bread from unacceptable (coarse, central hole or collapsed) without TGase to quite uniform with TGase. The authors identified protein crosslinking and possibly also deamidation as responsible for these effects of TGase. Nevertheless, the overall low volumes suggest that no protein structures with a gas holding capacity comparable to wheat gluten were formed.

In order to understand the limited success with TGase in gluten-free breads, we first have to address wheat bread. The decisive critical question to ask would be, whether wheat gluten is really crosslinked. Although there is still a lot of discussion among cereal scientists about the nature of gluten, there is also at least some consensus. Gluten is not a material dominated by covalent crosslinks like (vulcanized) rubber. Otherwise, dough could not be permanently deformed, as for example in case of sheeting, but would inevitably assume its original shape after the outer force is removed. Instead, it appears that the formation of high molecular, linear aggregates from glutenin subunits via disulfide bridges is decisive in wheat gluten. This can be concluded, because rheological dough properties (resistance to extension) were highly correlated to x-type HMW glutenin subunits, which tend to form linear polymers, but not to y-type subunits, which tend to form covalent crosslinks (Belitz et al., 2004). It is also important to remember, that gluten contains gliadin, which remains monomeric and acts as lubricant for the aggregated glutenins. Analogies from polymer science suggest that the linear glutenin polymers are linked to each other only via transient, non-covalent crosslinks, so-called 'entanglements' (Singh and MacRitchie, 2001).

Thus, the one important question for the use of TGase would be whether, at sufficiently low dosage, this enzyme would be able to form large, predominantly linear protein aggregates out of non-gluten proteins (equivalent to glutenin polymers). At the same time, a considerable portion of the proteins should remain unchanged or only aggregated to a small

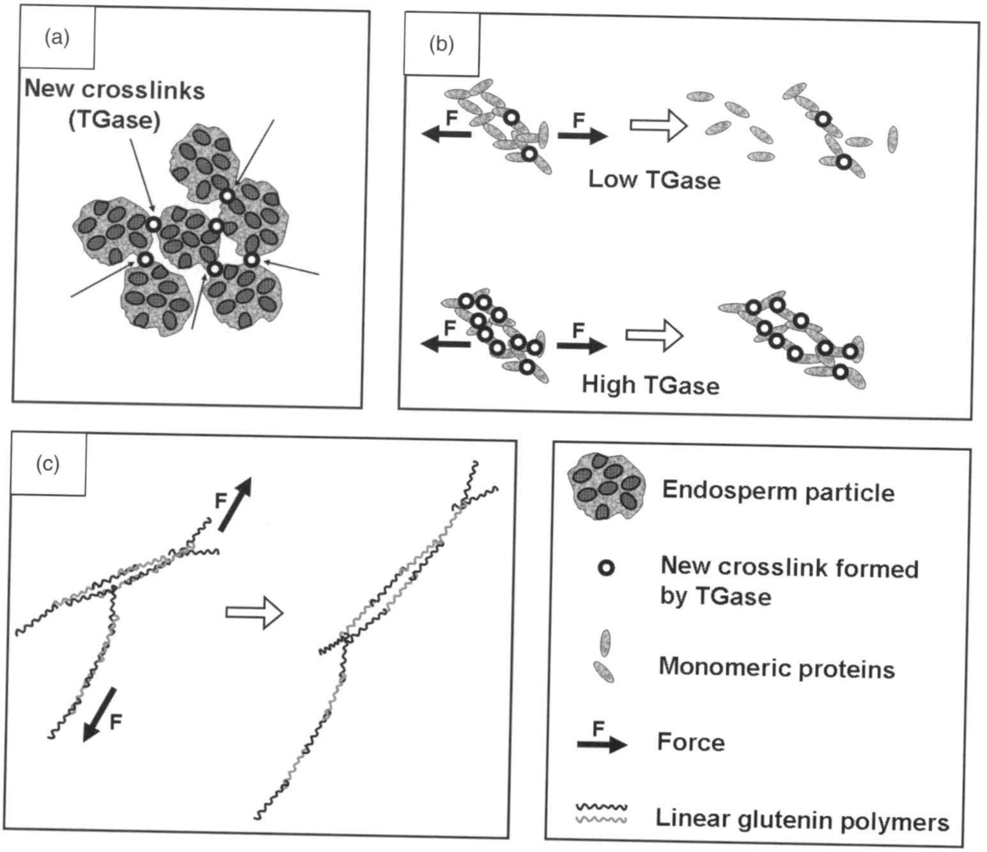


Fig. 9.11 Models for the effects of transglutaminase (TGase) on proteins in gluten-free cereals (a, b). (a) Flour particles (endosperm particles) glued together at their surfaces. (b) Soluble monomeric proteins with low and high dosage of TGase. (c) Wheat gluten for comparison (linear glutenin polymers forming entanglements).

degree (so that we have something that functions like gliadin). This is a considerably more complex question than whether this enzyme can create 'crosslinks' or 'networks'. Another prerequisite for the formation of a gluten-like polymer would be that the storage proteins are accessible. If they are encapsulated in protein bodies, the formation of an extended network through the whole dough (i.e. before baking!) appears impossible. At best, we can then stick protein bodies or endosperm particles together via their surfaces. Models for the effects of TGase (not leading to a gluten-like network) are suggested in Fig. 9.11, and a model for wheat gluten is given for comparison. It must be emphasized that these models solely explain why TGase can most likely not create artificial viscoelastic gluten. Whether it may possibly have other beneficial effects, like improving the ability of proteins to stabilize the gas–liquid interface in bubbles or increasing the cohesiveness of otherwise crumbly gluten-free bread, is not addressed in this figure. These issues require further research.

It has been pointed out before that a certain risk for celiacs is associated with the use of TGase (for details, see Section 9.2.3.3).

Summary: *Up to now, the use of transglutaminase (TGase) has not resulted in the development of greatly improved gluten-free bread. It appears that no protein network with viscoelastic properties similar to gluten could be produced. According to our opinion, the assumption that any protein network will improve gluten-free bread is not sufficiently supported by theoretical considerations and experimental data. More attention has to be paid to the nature of the protein network, and the details of the structure of wheat gluten have to be taken into account.*

9.3.7 Wheat bread, rye bread and gluten-free bread

9.3.7.1 *Wheat-like viscoelasticity of zein dough*

We have discussed some aspects of the functionality of wheat gluten in the previous section. In short, linear glutenin polymers form entanglements and thus contribute elasticity. Gliadins remain monomeric and act as a lubricant for the glutenin polymers. The use of TGase was one attempt to create viscoelastic protein networks from storage proteins of gluten-free cereals. Another was described by Lawton (1992). Isolated zein, maize starch and water could indeed form a viscoelastic dough, provided that they were mixed at elevated temperatures (e.g. 35°C), above the glass transition temperature of zein (around 28°C, Lawton, 1992). Such dough had a protein fiber network, which could be visualized by scanning electron microscopy (Lawton, 1992). Studies in our lab (Schober et al., 2008) showed that addition of HPMC to such zein-starch dough yielded well-leavened bread. The resulting dough was less elastic than wheat dough, but could be handled similar to the latter. For example, it could be rolled into strands and these could be slung into the shape of pretzels. Zein dough might therefore have its niche for the production of gluten-free products other than pan breads (hearth-type breads, braided breads, soft pretzels and various types of rolls). A technological challenge is that zein dough must not be cooled below zein's glass transition temperature. We could overcome this problem by preparing it at 40°C, that is, close to the maximum temperature that regular baker's yeast can tolerate. Then, it would not easily cool below glass transition while being worked at room temperature (e.g. into pretzels). Obviously, all proofing steps must also be carried out at elevated temperatures.

Summary: *Viscoelastic dough can be made from zein (maize prolamin), water and added starch by mixing at elevated temperatures (e.g. 35–40°C). Such zein dough might have its niche for specialties like hearth-type breads, braided breads, soft pretzels and rolls.*

9.3.7.2 *Pitfalls when determining viscoelasticity*

Frequently, dynamic oscillatory tests are used to determine the viscoelastic behavior of dough or batter. Such tests are easily misinterpreted. An example would be as follows:

We have shown the effects of different water contents on sorghum bread above (Section 'The right balance between ingredients', p. 151) and in Fig. 9.7. While 105% water on a flour-starch basis resulted in acceptable bread, 80% produced very dense, hardly leavened bread. We studied both batters, omitting the yeast, by dynamic oscillatory frequency sweeps in the linear viscoelastic region (Fig. 9.12). Unexpectedly, the phase angles were very low (10–15°) for both batters. (For any material, the phase angle is between 0° and 90°, where

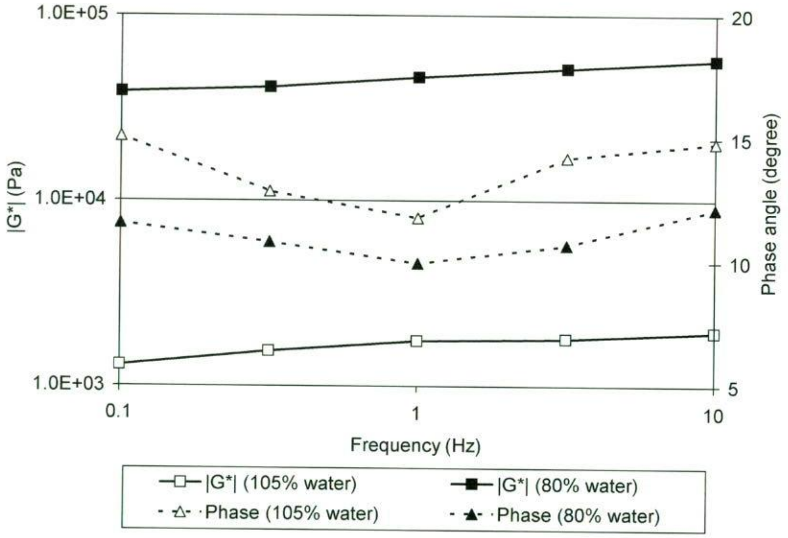


Fig. 9.12 Fundamental rheological properties of the sorghum–maize starch batters from Fig. 9.7 (yeast omitted). The absolute value of the complex dynamic modulus ($|G^*|$) was significantly different between the two samples for all measured frequencies ($P < 0.05$), the phase angle only at 0.1 Hz ($P < 0.05$). Measurements were done in triplicate. (Parallel, serrated plates, 25 mm diameter, target strain 5×10^{-4}, gap autoadjusted around 3 mm to reach a normal force target of 0.01 N, temperature 30°C.)

0° corresponds to ideal elastic behavior, and 90° to ideal viscous behavior). For comparison, wheat doughs measured under similar conditions in the linear viscoelastic region showed phase angles between 20° and 24° in the same frequency range (Clarke et al., 2002, 2004). Can we thus conclude that our sorghum batters are more elastic or have stronger 'networks' with more covalent crosslinks than wheat dough? Obviously not, because when just manually evaluating the sorghum batters, the batter with 105% water showed liquid-like behavior, that is it flowed without regaining its original shape, while the low water content produced a dough-like product, that however was brittle and broke upon deformation in the centimeter range, rather than regaining its original shape after being deformed. (Ideal elastic means by definition that a body regains its original shape immediately after the deforming force is removed.) So, what is the cause of this apparent contradiction? Dynamic oscillatory tests in the linear viscoelastic region are done at very small deformations. Let us assume that we have a cube that is sheared with a strain of 5×10^{-4}. Shear strain (γ) is defined as displacement of the top of the cube (x) divided by its height (h):

$$\gamma = \frac{x}{h}$$

Thus, a strain of 5×10^{-4} at a height of 3 mm as in measurement in Fig. 9.12 would correspond to a displacement of 1.5 μm. This is smaller than all common starch granules (Belitz et al., 2004) and obviously much smaller than most endosperm particles, which comprise starch granules embedded in matrix protein. Therefore, we measure various interactions, like interactions between starch granules or between endosperm particles or between starch and proteins. However, if there should be a gluten-like protein network, its properties would be masked by the abundant starch. Therefore, these measurements cannot clarify, whether there is a continuous gluten-like network or not. These interpretations have been put forward already more than a decade ago by Amemiya and Menjivar (1992) for wheat dough. Comparable to the present study, Parkkonen et al. (1994) found that rye dough had substantially higher elastic moduli (G') than wheat dough, which they attributed to larger size and rigidity of the rye particles. These interpretations fit well to the model for sorghum batter (Section 'Viscosity increase', p. 148 and Fig. 9.9). Damaged starch, swollen to a considerable size, endosperm and bran particles would stick together and result in an elastic response (therefore the low phase angles in Fig. 9.12). This is true, as long as the deformations are small enough, so that the particles are not torn apart, but can reset to their original configuration after removal of the force, similar to a weak network. When we extend our batter or gluten-free dough in the centimeter or possibly only millimeter range, their weak interactions are broken, and the response is no longer elastic (this is what we feel, when we manually assess these systems). A more appropriate test method in order to find out, whether a viscoelastic protein network is present, would be large deformation extension tests (e.g. extensigraph or Kieffer extensibility rig). If we are unable to form the dough strands required for these instruments, than we most likely have no continuous viscoelastic protein network through our dough or batter. If there is one, then we should get extension curves comparable to those for wheat dough.

An important rheological aspect of gluten-free batters is their viscosity (or, more accurate, apparent viscosity, a term that applies to non-Newtonian fluids). We saw in the section on rice, that an optimum water content exists (insufficient water levels resulted in stiff dough that rose only little, excessive water resulted in overexpansion and thus large holes in the breads, see Section 9.3.4.2). This would suggest that an optimum viscosity exists, which is determined by the water level and the properties of the flour and added hydrocolloid. Possibly, dynamic oscillatory tests could be used to determine this optimum (e.g. by defining an optimum absolute value of the complex modulus ($|G^*|$) at a certain frequency). Figure 9.12 indeed indicated that higher water (105%) resulted in a significantly lower $|G^*|$ over the whole frequency range than lower water (80%). (Lower $|G^*|$ means less resistance to deformation, or 'softer'). However, due to the sensitivity of this type of measurement to particle size and particle rigidity, and to interactions between various components like damaged starch, endosperm and bran particles, we cannot expect that a comparison of $|G^*|$ between different types of flours would work. We would therefore recommend large deformation measurements for the determination of an optimum (apparent) viscosity, so that particle size and short-range interactions become less important. Examples would be determination of the force required for extrusion (Schober et al., 2005), or standard methods like Newport Rapid Visco Analyser (RVA) or Brabender Amylograph (ICC Standards No. 162 and 126/1; ICC, 2000). The latter methods also measure viscosity increase upon heating and thus starch gel strength, and are widely used for the evaluation of rye. This leads to the next section.

Summary: *We cannot conclude from low phase angles in dynamic oscillatory measurements that gluten-like protein networks are present in a gluten-free dough. Low phase angles might also originate from interacting particles like starch, endosperm particles or bran. More useful are large deformation measurements (e.g. extension tests). For batters, the optimum viscosity, measured at large deformations, appears much more important than dynamic properties, and we recommend extrusion tests, Amylograph or Rapid Visco Analyser.*

We should be very careful when claiming that in gluten-free breads, 'elasticity' or 'networks' are required. We need a clear definition (elasticity: in which deformation range; what kind of networks).

9.3.7.3 *The analogy between rye breads and gluten-free breads*

At the beginning of this section, we have to emphasize that rye is not suitable for celiacs (see Section 9.2.2). However, we want to include it in the discussion, because it helps to understand gluten-free bread. It is also important to emphasize that we relate to a type of rye bread, which is made from at least 90% rye flour. Such bread is popular in Central, Eastern and Northern Europe. Its production has been described by Meuser et al. (1994) and Seibel et al. (1978). Such rye bread is distinctly different from rye bread sold for example in the USA, which contains mainly wheat and only a comparatively small portion of rye in order to modify flavor and appearance (Hoseney, 1998).

When comparing gluten-free batters, rye doughs and wheat doughs, gluten-free batters resemble rye dough much more than wheat dough. Gluten-free batters and rye dough are highly viscous, but have little elasticity and extensibility upon deformation in the centimeter range. Rye dough and those gluten-free batters made with hydrocolloid addition both leave a 'slimy' feeling at one's hands. On a more scientific level, both are characterized by a bubble structure not supported by a continuous viscoelastic gluten network. Water binding and dough cohesion are achieved by natural hydrocolloids in rye (pentosans and to a lesser degree β-glucans, Parkkonen et al., 1994), while in gluten-free batters, added hydrocolloids like xanthan gum and HPMC play an important role. These substances also cause the 'slimy' feeling just mentioned. HPMC and soluble pentosans have in common that they are surface active and may therefore stabilize gas cell walls (see Sections 9.3.1.2 and 'The stabilization of bubbles', p. 150).

During baking, in wheat bread, besides starch gelatinization, the denaturation of gluten contributes to crumb setting. In rye and gluten-free breads, it is largely the starch that causes crumb setting, first by gelatinization, later by amylose retrogradation (see Section 'Emulsifiers, starch and crumb properties', p. 150). Therefore, excessive degradation of starch has to be avoided. This is done by acidification in rye bread (sourdough or added acids), so that the dough pH is well below the pH optimum of the α-amylase. This pH optimum is about 5.5–5.7 (Belitz et al., 2004). In gluten-free bread, we have to take care that we do not overdose added amylolytic enzymes. This point is relevant for the next section.

The role of proteins in rye bread and (egg-free) gluten-free breads is generally most difficultly to understand. The idea that only starch and pentosans are responsible for rye dough and crumb formation is an over-simplification. Parkkonen et al. (1994) reported that proteins play an important role in the rye dough structure directly after mixing, when degradation of cell walls is still limited and therefore not enough pentosans have been released into the dough. Fluorescence microscopy, in which cell walls and proteins had been specifically stained, revealed that rye dough directly after mixing contained unbroken bran, aleurone, endosperm particles and starch granules dispersed in a protein matrix. The protein content of the flour appeared to be a decisive factor for the continuity of the protein matrix. In apparent contrast, Tuukkanen et al. (2005) concluded that proteolytic breakdown of rye proteins (mainly secalins) during sourdough fermentation may have a key role in rye breadmaking. These authors mentioned various aspects of proteolysis, like the possibility that soluble rye protein structures might stabilize foams, or that small peptides and free amino acids might act as flavor precursors and nutrients for the microorganisms in sourdough. The situation in gluten-free breads is similarly controversial. We have mentioned a possible positive role of soluble proteins on gas cell stabilization, and a negative role of protein aggregation during baking in the section on sorghum bread (Section 'The role of proteins and protein networks', p. 151). Some degradation of sorghum proteins during sourdough fermentation was therefore beneficial, as it prevented this aggregation upon baking (see Section 9.3.3.2 and Fig. 9.8). In yet unpublished experiments, we also tried to degrade the sorghum proteins completely, using a high dosage of an endo-protease. However, the resulting bread was of very low volume and had a sticky crumb, suggesting that a partial, but not a complete protein degradation is required. Thus, it appears that proteins have some role in the crumb formation of gluten-free batter breads. This is also in agreement with results of studies like Gujral and Rosell (2004), where TGase showed some beneficial effects in combination with rice flour and HPMC. As TGase acts upon proteins, its effect on bread quality shows that proteins must play a certain role. Clearly, more research is required to identify the exact role of proteins in these types of gluten-free breads.

Summary: *Gluten-free breads and rye breads (from >90% rye flour) resemble each other technologically, as they are both made from soft, batter-like doughs and contain natural or added hydrocolloids rather than a continuous, viscoelastic gluten-network (pentosans and β-glucans in rye vs. added xanthan gum and HPMC). It appears that this analogy has been largely ignored in the literature. There might be a chance for a better understanding of both systems, if results from both areas were compared, and the analogies might be exploited for the improvement of bread quality.*

(Rye bread serves as a technological model only, it is not safe for celiacs!)

9.3.8 Staling

Quick staling – or increase in crumb firmness – is one of the most unpleasant properties of gluten-free bread. The mechanisms of staling are still being debated. Historically, it has been associated with starch retrogradation. However, more recently, for regular wheat bread the involvement of gluten in the staling process has been suggested, in a way that starch might interact with gluten fibrils and crosslink them (Martin et al., 1991; Hoseney, 1998). Meanwhile, this model has been questioned again. At least, it has been reported, based on a comparison of regular wheat bread and gluten-free starch bread, that interactions between starch and gluten are not essential for the crumb firmness increase. Starch retrogradation alone is sufficient to cause bread firming (Morgan et al., 1997). In gluten-free bread, we should focus on the starch alone. Within the starch phase, amylose retrogradation occurs very fast upon cooling and helps to stabilize the crumb. In contrast, amylopectin retrogradation is slower and seems to be the decisive factor for aspects of staling like crumb firming and loss of elasticity (Belitz et al., 2004). For pure starch breads from potato or wheat starch, Keetels et al. (1996) suggested a detailed model for crumb structure and staling (see Section 9.3.1.3).

It is known that crumb firmness increases over storage time also when no drying occurs (Hoseney, 1998). Nevertheless, drying doubtlessly speeds up the perceived firmness of bread. Hydrocolloids, including xanthan gum and HPMC, have been shown to reduce moisture loss in wheat bread that was stored unpacked (Guarda et al., 2004). HPMC, but not xanthan gum, lowered the increase in crumb firmness over 24 h of storage in the same study, and the authors assumed that HPMC might inhibit amylopectin retrogradation by binding to the starch.

Shortening and emulsifiers (monoglycerides) are widely used in wheat bread to delay staling (Hoseney, 1998; Belitz et al., 2004). Several mechanisms for their antistaling effect have been suggested. Both substances might limit starch swelling (Martin et al., 1991; Hoseney, 1998), and therefore subsequently starch interactions that would lead to staling. This is essentially the same argument used above to explain, why these substances soften or weaken the crumb (Section 'Emulsifiers, starch and crumb properties', p. 150). As before, we have to carefully balance between desirable prolonged softness of the crumb and undesirable crumb weakening. It has also been reported that during baking, emulsifiers (in this case, monoglycerides) form complexes with amylose and amylopectin, retarding retrogradation (Belitz et al., 2004). Again, it is obvious that we should not prevent amylose retrogradation too much, because we would otherwise prevent the proper setting of the crumb.

Use of bacterial α-amylases is a well-established method to delay staling in wheat bread (Martin and Hoseney, 1991). Thermostability of these amylases is a critical factor. There is a so-called 'window' for the amylase activity. It starts upon starch gelatinization, because ungelatinized starch (unless mechanically damaged) is not notably attacked by amylases, and ends upon thermal inactivation of the amylase (Martin and Hoseney, 1991). While bacterial α-amylases are generally relatively heat stable (Akers and Hoseney, 1994), it might be desirable to select such enzymes with only intermediate temperature stability to avoid excessive starch degradation and dextrin production (Gerrard et al., 1997). This appears especially important in gluten-free bread due to the important role of starch.

There is considerable debate in the literature about how these α-amylases delay staling. While there is agreement that α-amylases produce specific mixtures of dextrins from starch, there is no consensus whether these dextrins are the cause of the delayed staling, or whether they just reflect the degradation of starch. In the latter case, the modification of the starch itself would be the cause of the delayed staling and the dextrins just a symptom. Martin and Hoseney (1991) and Akers and Hoseney (1994) suggested that dextrins of a certain size are the cause of delayed staling, for example, by interfering with crosslinks between gluten and starch in staling wheat bread (Martin and Hoseney, 1991).[2] Gerrard et al. (1997) and Morgan et al. (1997) disputed this view and put forward the hypothesis that the modification of the starch itself is the cause and dextrins just indicate this modification.

One bacterial α-amylase of intermediate temperature stability (Novamyl® by Novozymes, Switzerland) that has considerable potential in delaying firming of the crumb has been used by Gerrard et al. (1997) in regular wheat bread and by Morgan et al. (1997) in gluten-free starch bread.

Summary: *Staling is a large problem in gluten-free bread. We can slow it down by adding hydrocolloids (especially HPMC), shortening and emulsifiers and/or bacterial α-amylases. In the case of shortening and emulsifier, overdosage may easily weaken the crumb, and we also have to pick the right emulsifier (which may involve trial and error). In a similar way, bacterial α-amylase may destroy the crumb structure by excessive starch degradation, therefore we should take care that we do not select an excessively thermostable enzyme and that we do not overdose.*

9.4 CONFECTIONERY PRODUCTS

9.4.1 General

In most confectionery products, excluding puff pastry and sweet yeast leavened breads, full gluten development is undesirable and instead egg, fat and/or sugar play an important role for the physical structure. Therefore, the problems in producing these products from gluten-free flours are small. The technological steps for a successful production can mostly

[2] This interpretation would obviously require modification when applied to gluten-free bread, in a way that dextrins would interfere with starch-starch interactions.

be derived from wheat-based formulations. Other aspects, like desirable color or flavor, become more central questions. These, however, depend largely on the consumers' taste in various countries and are beyond the scope of this book chapter. We therefore want to limit the following sections to a small number of examples, where technological problems were encountered.

9.4.2 Gluten-free cakes

As with all bakery products, cakes vary between countries. Hoseney (1998), with a US background, differentiates layer cakes (high ratio, i.e. more sugar than flour), angel food cakes (based on foam from egg white and sugar, only little flour) and pound cakes (heavy, rich cakes). For the present section, we focus on layer cakes, as described in AACC Standard 10–90 (AACC International, 2000). The formulation is (on a flour basis) 100% flour, 140% sugar (saccharose), 50% shortening, 12% non-fat dry milk, 9% dried egg whites, 3% salt (NaCl), optimum (typically 125–145%) water and baking powder. The procedure comprises sifting of dry ingredients, addition of shortening and part of the water, mixing in several steps with addition of the remaining water, scaling into pans and baking.

Although the study of Glover et al. (1986) addressed wheat–sorghum composite flours (obviously not appropriate for celiacs and people with wheat allergies), several important principles for gluten-free cake production can be derived. The basic problem was that cake volume decreased as the percentage of sorghum increased in the sorghum–wheat flour mix. At high levels (30–50% of sorghum), crumb became very brittle.

Problems associated with the sorghum flour were related to large particle size, lack of polar lipids, especially glycolipids, and high starch gelatinization temperature. The high gelatinization temperature resulted in a high percentage of ungelatinized starch. High gelatinization temperature and lack of glycolipids are in agreement with the literature (Chung and Ohm, 2000; Lineback, 1984). Glover et al. (1986) could improve the cakes by technological means. Finer milling, using a pin mill, resulted in smaller particle size and higher starch damage. The water binding and resulting increase in batter viscosity due to starch damage appear to be desirable specifically in high ratio cakes (Evers and Stevens, 1985) and pin milling improved cake quality to a limited degree. Use of glucose instead of saccharose in the recipe improved cake volume, crumb grain and crumb texture considerably. A higher degree of starch gelatinization was found as a consequence of the use of glucose. Spies and Hoseney (1982) reported that sugars delay starch gelatinization and that saccharose has a stronger effect than glucose. They attributed these findings to two effects of the sugars: The first effect would be lowering the water activity, that is, less water is available for starch gelatinization because it is bound by the sugar. The second effect would be interactions between sugars and starch. Sugars would bind to the starch chains and promote their interactions, that is, the sugars would act as bridges between starch chains. Longer sugar molecules could be expected to be more efficient in forming such interactions and bridges. The longer saccharose molecule (disaccharide) would therefore increase starch gelatinization to a larger degree than glucose (monosaccharide). Use of glucose instead of saccharose in the cake would cause the starch to gelatinize earlier. Similar to sorghum, rice is generally characterized by a high starch gelatinization temperature (see Section 9.3.4.1). We could therefore expect similar problems with rice flour as with sorghum flour. In (wheat-based) layer cake baking tests with reconstituted flours, where in a commercial cake flour wheat starch had been replaced by a variety of other starches (rye, barley, maize, rice and potato), rice starch did indeed perform worst (Sollars and Rubenthaler, 1971).

If in a grain like sorghum, the content of natural emulsifiers (i.e. polar lipids) is insufficient, emulsifiers should be added to the batter. In layer cakes, propylene glycol monostearate is widely used to facilitate air incorporation (Hoseney, 1998).

Summary: *It appears that the following traits of a gluten-free flour are undesirable for layer cake production: large particle size, insufficient content in polar lipids and high starch gelatinization temperature. Beneficial are fine milling of the flour, and addition of emulsifiers to compensate for a lack in polar lipids. For a given flour with a given starch gelatinization temperature, exchange of sugars is possible, and use of glucose instead of saccharose may lower the starch gelatinization temperature in the batter. However, we might better try to select gluten-free flours with lower starch gelatinization temperatures.*

9.4.3 Gluten-free biscuits

Biscuits (British English, equivalent to 'cookies' in American English) are based on formulations high in sugar and shortening, but relatively low in water (Hoseney, 1998). They can be produced using either rotary molds, or by sheeting and cutting, or by extrusion through an orifice and cutting. Important are a tender bite of the biscuits and their size. Size (width and height) is important in industrial production because the biscuits have to fit into their boxes, and is governed by the amount they spread during baking (Hoseney, 1998). It has been furthermore pointed out that damaged starch is undesirable in biscuits, because it binds water. Since the baked biscuits are very low-moisture products, this extra water has to be evaporated during baking, increasing the required energy (Evers and Stevens, 1985). A different argument would be that the degree of starch damage affects how much the biscuits spread. The higher the starch damage the more water from other ingredients is bound and consequently the spread is lower (Thomas and Atwell, 1999). At least for formulations with high sugar and low water (e.g. 60% sugar and about 23% water on a flour basis), little or no starch gelatinizes during biscuit baking due to limited water availability (Abboud and Hoseney, 1984). We could therefore conclude that, in contrast to cake, starch gelatinization temperature is relatively unimportant in biscuits. However, upon baking shortening melts and sugar dissolves, which increases fluidity and thus allows the biscuits to spread (Abboud and Hoseney, 1984; Hoseney, 1998). The latter author furthermore assumed that proteins might play a role in controlling viscosity during baking and thus biscuit spread as starch is not gelatinized. This might require a more careful selection of the flour mixture in gluten-free cookies. A successful formulation for a sheeting and cutting procedure used brown rice flour (70%), corn and potato starch (10% each) and soy flour (10%) in combination with egg (Schober et al., 2003).

A study on biscuits from sorghum and pearl millet covered many of the aspects just mentioned and is therefore a good example for problems encountered in gluten-free biscuit development and finding of solutions (Badi and Hoseney, 1976). These biscuits were produced following the micro method of Finney et al. (1950). The formulation (on a flour basis) was flour (100%), sugar (saccharose, 60%), shortening (30%), $NaHCO_3$ (1%), NH_4HCO_3 (0.75%), non-fat milk solids (3%), salt (NaCl, 1%) and water (to optimum). The procedure involved creaming of sugar, shortening, non-fat milk solids, salt, leavening agents and water. Finally, flour was added with very short mixing. Sheeting, cutting and baking followed. According to Finney et al. (1950), sufficient spread and a well-broken top with numerous small cracks are desirable for these biscuits. In contrast, sorghum and millet biscuits produced by Badi and Hoseney (1976) with the exact procedure of Finney et al. (1950) lacked spread and top cracks and were, according to the authors, 'tough, hard, gritty and mealy'.

Badi and Hoseney (1976) could identify several problems associated with the sorghum and millet flours, including the lipid composition, and a high degree of starch damage. Improvement of the biscuits was possible by several steps: The first step was adding emulsifiers (unrefined soy lecithin or refined lecithin plus monoglycerides), which improved top grain and spread. This is in line with the lack of polar lipids in sorghum described above and in the literature (Chung and Ohm, 2000). Next, incubation of the sorghum or millet flours with malt syrup or just water for several hours and air drying was done to remove damaged starch. This improved spread and top grain even more, and also reduced grittiness. The authors suggested that only malt treatment removed damaged starch, however, it would appear plausible that incubation with water could also reduce the amount of damaged starch due to the action of grain amylases. (It should be kept in mind that barley malt is not gluten-free, thus it should be replaced by microbial amylases in a gluten-free formulation.) Grittiness could be further reduced by increasing the pH of the biscuit dough (use of Na_2CO_3 instead of $NaHCO_3$). Our own data suggest that sorghum proteins can be solubilized under alkaline conditions (Fig. 9.13). Therefore, we might assume that the protein matrix of endosperm particles is dissolved as the biscuit dough gets more alkaline, thus eliminating these particles and reducing grittiness. Finally, Badi and Hoseney (1976) addressed the remaining problem of fragility of the biscuits by blending the gluten-free grains with wheat. As this step is impossible in gluten-free biscuits, we would suggest to try egg addition if gluten-free biscuits are too fragile.

Summary: *Gluten-free flours for biscuit (cookie) production should have low starch damage, while starch gelatinization temperature appears to be less important because starch tends to not gelatinize in biscuits anyhow due to high sugar and low water concentrations. Controlling the biscuit spread is important in the industrial production, so that the biscuits fit in their boxes. Starch damage, water level and possibly proteins from flour or added egg affect the spread.*

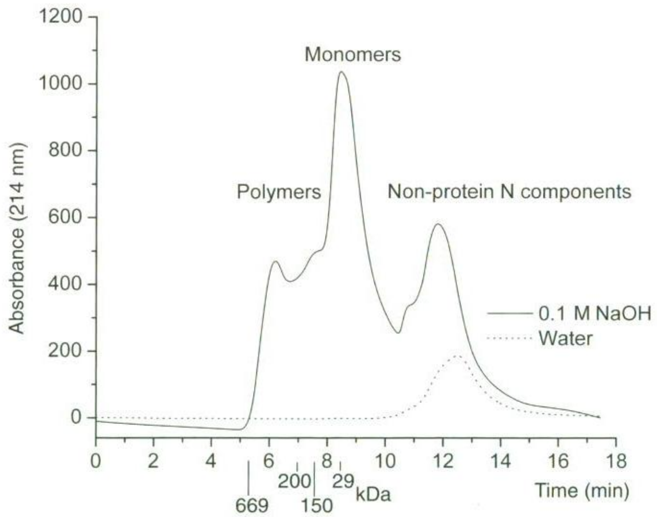


Fig. 9.13 Size-exclusion HPLC of sorghum flour extracted for 15 h with water or 0.1 M sodium hydroxide solution (100 mg flour plus 400 μl liquid). Separation on a Phenomenex® BioSep-SEC-S 3000 column (300 × 7.8 mm), mobile phase 50% acetonitrile in water plus 0.1% TFA, flow rate 1.0 ml/min, column temperature 40°C, 15 μl injection, detection at 214 nm.

9.5 CONCLUDING REMARKS

We know that this chapter contains a lot of information. We are also sure, that some of it might be too simplified, too complicated, too detailed or not detailed enough, depending on you, our reader and your specific background and needs. We hope that you could benefit from some of the information provided and that the literature cited will provide additional help. If you are new in the area of gluten-free bread, do not be too easily discouraged. People who are used to wheat bread will sometimes be quite critical about gluten-free bread – but we have to advance step by step, improving quality, healthiness and maintaining safety of the products.

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